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MANGANESE TOXICITY IN STANDARD CULTURE SOLUTIONS

by D. EMERTON WILLIAMS and JAMES VLAMIS

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Barley plants grown in standard Hoagland culture solutions in which the potassium concentration was varied from 0.01 to 50 ppm invariably developed a large number of dark brown necrotic spots. The spots appeared on the tips of the cotyledons from one to two weeks after 5-day old seedlings were placed in culture solutions. The spotting progressed down the leaf until the entire leaf surface was covered and did not appear to be related to any potassium treatment. As the plants grew the necrosis spread through the plant developing in turn on the 1st, 2nd, 3rd, *etc.* leaves. New leaves seldom showed necrotic symptoms. Barley plants grown in 100-litre tanks with one-fifth the normal Hoagland concentration of macrosalts showed severe spotting. In previous experiments by Dr. Albert Ulrich of the Plant Nutrition Department at Berkeley, the appearance of the spots had been reduced by the addition of silicate to the culture solution. However, the actual cause of the spotting and the role of the silicate were unknown.

Three possible causes for the necrosis appeared to be: 1. Silica, as indicated by Ulrich's work, was an essential element and the spots were a manifestation of Si-deficiency. 2. The symptoms, similar to those which have been observed on barley plants grown in acid soils [4] and in high-Mn culture solutions [1] might be the result of a toxic concentration of manganese (Mn) in the culture solution. 3. The barley plants exhibited margin and tip burn as well as spotting and the necrosis might be due to a toxic quantity of boron (B) resembling symptoms observed on barley grown on high boron soils.

To investigate these three possibilities a total of nine experiments were carried out in the soils and plant nutrition greenhouses at

Berkeley. The 1st experiment was started in April of 1953. The final experiment was concluded in November of 1954.

EXPERIMENTAL

A preliminary test carried out from April 22 to June 15, 1953 showed that increasing the concentrations of Mn or B increased the amount of necrosis. Decreasing the Mn- and B-concentrations decreased the toxic symptoms, as did the addition of silica to the culture solution.

In the 5 experiments whose data are reported in Figures 1 and 2 five one-week old seedlings germinated in distilled water were placed in 45 liters of culture solution and grown for periods of 6 weeks duration. The culture solutions used were one-fifth strength and full strengh Hoagland solutions[2]. Hoagland's micro-nutrient solution containing Mn, B, Cu, Zn, and Mo was added at 0, $^1/_{20}$, $^1/_5$, and full strength. Iron was added twice weekly in the form of low manganese $FeSO_4$ at a concentration of 0.1 ppm. The plants were harvested, dried, and weighed. Where possible the plant material was separated into 3 grams of old leaf tissue, 3 grams of new leaf tissue, remaining leaves, and roots. Each fraction was digested in HNO_3 and analyzed colorimetrically for manganese following the development of the permanganate color using periodate in the presence of phosphoric acid.

In Fig. 1 the ppm of Mn found in the leaf tissue at the time of harvest is plotted against the concentration of Mn initially present in the culture solutions. The two solid curves represent the amount of Mn found in old leaf tissue. The upper curve represents data obtained when the macro-salts are present at $^1/_5$ the normal Hoagland concentrations, the lower curve when the macro-salts are present at full Hoagland concentrations. The two dotted curves represent the Mn found in the new leaf tissue of the same plants. The amount of Mn in the leaf tissue was found to increase with increasing Mn in the culture solution. Old leaves contained much more Mn per unit weight of tissue than did the young leaves and in contrast were highly spotted.

It is obvious from the data in Fig. 1 that a decrease in the concentration of minor elements in solution is associated with a decrease in the Mn-content of the leaf. Further a decrease in the Mn-content

of the nutrient solution resulted in a general decrease in the Mn-content of all portions of the plants including the roots. A similar decrease in the Mn-content of the plant can be observed when the concentration of macro-salts is increased.

In Fig. 2 the Mn-content of the old leaf tissue is plotted as a function of the concentration of macro-salt in the nutrient solution. The macro-salt was varied at $^1/_5$, full, and double the usual Hoagland concentrations. The top curve represents nutrient solutions in

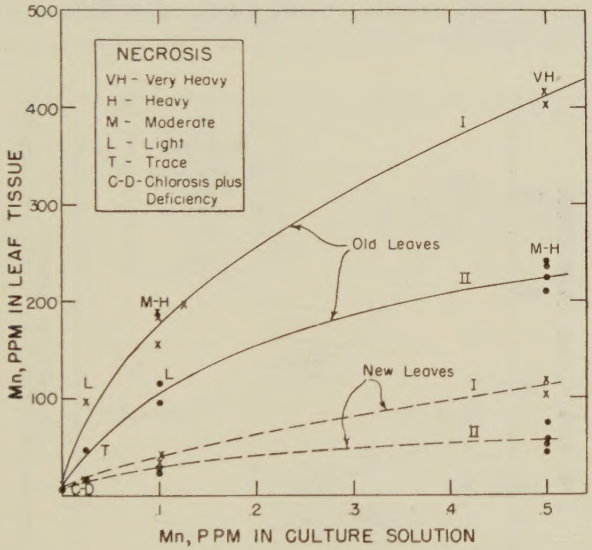

Fig. 1. Manganese content of new and old leaf tissue as influenced by the concentration of manganese in the culture solution. Macro-salts added at one-fifth (I) and full (II) Hoagland concentrations

which the micro-nutrient concentration (A5) is held at full Hoagland·strength. In the middle curve A5 is decreased to $^1/_5$ and in the lower curve to $1/_{20}$ of the standard concentrations. As was seen in Fig. 1 a decrease in A5 brought a decrease in Mn absorbed by the plant. Likewise an increase in the macro-salt concentration caused a large decrease in the Mn-uptake by the plants.

The symbols *VH*, *H*, *M*, *L* and *T* placed near the plotted values in Figures 1, 2 and 4 represent the degree of spotting observed on leaf material. Very heavy spotting was noted at 400 ppm Mn in the leaf (with full A5 in the culture solution) and moderate to heavy spotting

was observed at 200 ppm of Mn in the leaf tissue. The concentration of Mn in the new leaf tissue seldom exceeded 100 ppm when the recommended concentration of A5 was used and no necrosis was observed in the young leaves. The close correlation between the severity of spotting and the concentration of Mn in the old leaf tissue indicated that the necrosis might be due to a toxic quantity of Mn. However, since all of the 5 micro-nutrients were present in the solution and were decreased in the same proportion, it could not be assumed that Mn was the cause of the toxicity merely on the basis of a correlation between leaf spotting and Mn-content of the leaf tissue.

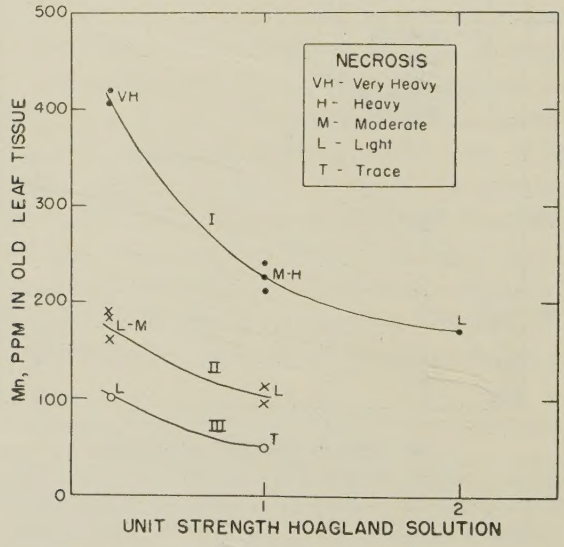

Fig. 2. Manganese content of old leaf tissue as influenced by the concentration of macro-salts in the culture solution. Micro-nutrient concentrations full (I), one-fifth (II), and one-twentieth (III) the recommended Hoagland concentrations.

It was observed that only light to trace spotting occurred when the A5 concentration was decreased to $^1/_{20}$ of the normal value. To separate the possible effects of toxicity due to Mn and B from that of the other minor elements, plants were grown in $^1/_5$ Hoagland solution with molybdenum (Mo), Zinc (Zn), and copper (Cu) maintained at $^1/_{20}$ the Hoagland concentrations and with Mn and B varied separately. The top curve of Fig. 3 shows the effect of B on

yield and toxicity symptoms when Mn is held at 0.025 ppm and B is varied at 0.1, 0.5, and 25 ppm. At a concentration of 0.5 ppm the plants were vigorous but showed marginal and tip burn. An increase in the B-concentration to 25 ppm caused a sharp drop in yield and a very severe leaf burn with large blotchy areas. At 0.1 ppm of B only light spotting with a small amount of tip burn was observed.

The lower curve of Fig. 3 shows the effect on yield and symptoms of increasing Mn from 0 to 0.1, 0.5, and 25 ppm with B maintained at 0.1 ppm. The plants at 25 ppm of Mn were heavily spotted and the yield was small. The symptoms observed were strikingly different from those obtained with B. The spots were smaller and looked like

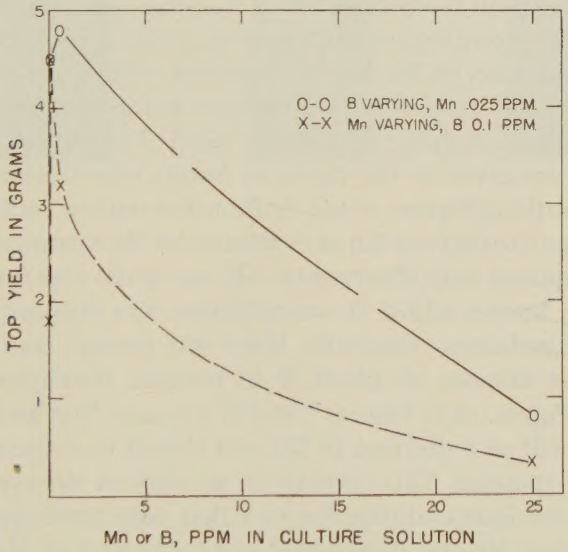

Fig. 3. Barley yields obtained at varied manganese or boron concentrations. Copper, molybdenum, and zinc held at 0.025 ppm.

small freckles covering the entire leaf surface rather than the edges as in boron toxicity. At 0.5 ppm Mn the spotting was still heavy, but the yield was high. At 0.1 ppm Mn the yield had increased to a maximum, the Mn-type spots had disappeared and the plants exhibited only a small amount of tip burn and boron type spotting. A further decrease of Mn to 0 ppm brought a sharp decrease in yield with the development of chlorosis and a new type of spotting which was

different from either of the two toxicity patterns and was identical with grey speck of Mn-deficiency [3].

The photograph of barley leaves (Plate I) compares necroses due to Mn- and B-toxicities as compared with an unspotted leaf. The leaves were taken from plants which had been grown in standard culture solutions. The leaf with the small spots was grown in a solution containing 0.5 ppm of Mn and 0.025 ppm B. The leaf with the large spots was grown in a solution containing 0.5 ppm of B and 0.025 ppm Mn. The leaf which is not spotted is a control leaf grown with both Mn and B held at 0.025 ppm.

Fig. 4 shows yield curves at full (upper curve) and $^1/_5$ (lower curve) macro-salt concentrations when Cu, Mo, and Zn are held at normal levels and Mn and B are varied at 0, $^1/_{80}$, $^1/_{40}$, $^1/_{20}$, $^1/_{10}$, $^1/_5$, and full Hoagland concentrations. As was seen in Fig. 3, yield increased as the concentrations of Mn and B decreased with a maximum yield obtained at 0.025 ppm. A further decrease in the Mn- and B-concentration led to a a sharp decrease in yield. Toxicity or deficiency symptoms are given in the figure as letters placed near the yield values. A striking feature of the figure is the narrow range between deficiency and toxicity of Mn as evidenced by Mn-symptoms. B-deficiency symptoms were observed in only one of the experiments even though the known added B-concentration was dropped to 0 in a number of instances. Evidently there was enough contamination even in the absence of added B to prevent B-deficiencies from occurring. Again, as in Figures 1 and 2, it is seen that an increase in the macro-salt or a decrease in Mn and B lead to a decrease in the severity of spotting. This decrease in severity of the symptoms as Mn and B are decreased plus the fact that only trace spotting was observed when Mn and B were held at a concentration of 0.025 ppm and Cu, Mo, and Zn were varied at $^1/_{20}$ and full strength is evidence that the spotting is related not to the presence of the Cu, Mo, or Zn but to a toxic supply of Mn and B.

Despite the lack of B-deficiency symptoms the yield curve in Fig. 4 may be a manifestation of either Mn- or B-toxicities and deficiencies. Table I gives a comparison of relative yields obtained in 3 experiments. In each experiment the 100% yield value is that obtained when the Mn concentration is 0.5 ppm. In all of the experiments for which data are shown in the table the macro-salt concentrations were full strength. Of the minor elements Cu, Mo, and Zn

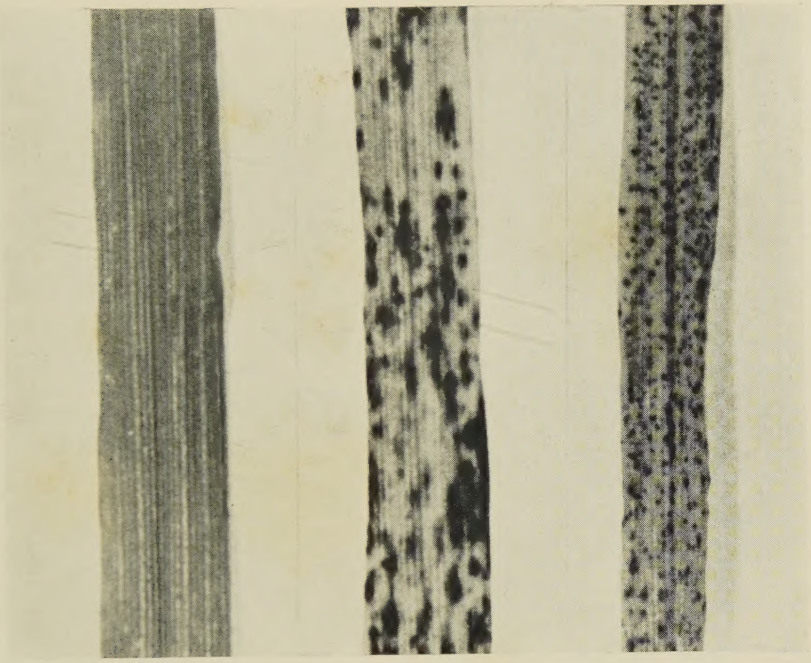

Plate I. Necrotic spots on barley leaves due to boron and manganese toxicities. Left to right: 1) normal unspotted leaf; 2) leaf exhibiting large blotchy boron spots; 3) leaf exhibiting small manganese spots.

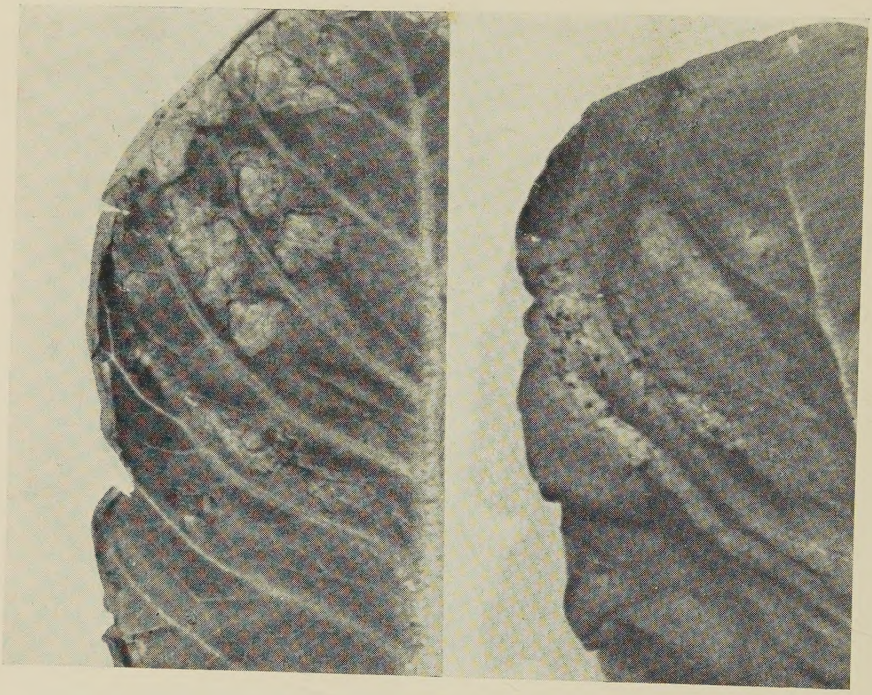

Plate II. Necrotic spots on lettuce leaves due to toxic quantities of boron and manganese. Boron toxicity exhibited in large necrotic areas on leaf at left, manganese toxicity in small spots shown on leaf at right.

were maintained at full concentration while Mn and B were varied either separately or together as indicated. Column 1) of the table shows the yield increasing to a maximum when both Mn- and B-concentrations are decreased from 0.5 ppm to 0.025 ppm. As the concentrations of both elements are decreased further a rapid decrease in yield is noted. At 0 concentration of Mn and B the yield is only 3.6% of the yield obtained when the concentrations were 0.5 ppm. Column 2) with B held at 0.025 ppm and Mn varying shows a maximum yield at 0.05 ppm with a lower yield at 0.025 ppm. Column 4)

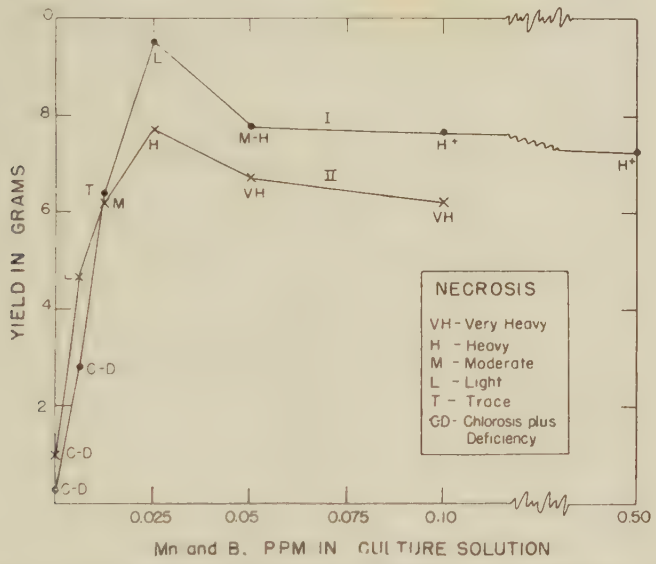

Fig. 4. Yield curves of shoots obtained at full (I) and one-fift (II) macro-salt concentrations with manganese and boron varied and copper, molybdenum and zinc held at full Hoagland concentrations.

where B is held at 0.5 ppm and Mn is varied shows a greatly reduced yield (5% of reference yield) at 0 ppm Mn. Columns 3) and 5), on the other hand, with Mn held at 0.025 ppm and 0.5 ppm respectively show no significant change in yield as the concentration of B is decreased. From the results of this table is it evident that changes in the concentration of Mn have a greater effect on the yield than do changes in the concentration of B. Evidently the shape of the curve in Fig. 4 is a manifestation of Mn concentration, with only slight yield variation due to B.

TABLE I

Relative barley yields with varying Mn- and B-concentrations					
Concentration of Mn and or B (ppm)	Experiment 5	Experiment 6		Experiment 7	
	1)	2)	3)	4)	5)
0.5	100*	100*	111	100*	100*
0.1	106	103	103	—	—
0.05	107	125	108	105	100
0.025	130	113	112	—	—
0.0125	88	—	—	—	—
0.00625	36	—	—	—	—
0.00	4	—	100	5	87

* In each experiment the 100% yield is that obtained when Mn is 0.5 ppm.
1) Mn and B varying together.
2) Mn varying, B = 0.025 ppm.
3) B varying, Mn = 0.025 ppm.
4) Mn varying, B = 0.50 ppm.
5) B varying, Mn = 0.05 ppm.

TABLE II

Percentage Mn removed by barley plants from nutrient solutions at 2, 4, and 6 weeks				
A*. Mn-concentrations variable				
Concentration of Mn in the solution (ppm)	Macro-salt concentration in Hoagland units			
	1/5	1/1	1/5	1/1
	4 weeks		6 weeks	
0.5	98	21	—	71
0.1	100	51	100	100
0.05	—	—	100	100
0.025	100	94	100	100
B*. Mn-concentration 0.50 ppm				
Growth period in nutrient solution	Macro-salt concentration in Hoagland units			
	1/5	1/1	2/1	
2 weeks	8	4	5	
4 weeks	48	30	26	
6 weeks	100	98	68	

* Experiment A conducted May to June 1953.
Experiment B conducted April to June 1954.

In Table II the percentages of total Mn removed by barley plants from culture solutions at 2, 4 and 6 week periods are shown. Manganese was found to be absorbed continuously from solution with almost 100% removal at 6 weeks. It is absorbed less rapidly from culture solutions where the macro-salt concentration is increased

TABLE III

Effect of Fe-source and Mn-treatment on barley yield and symptoms in the presence and absence of A4 (Cu, Mo, Zn, and B).

Iron and manganese treatment	Yield (g/plant)		Symptoms *	
	+A4	—A4	+A4	—A4
A. 1/6 Hoagland macro-salt concentration. Experiment 3				
Fe-tartrate, No manganese	—	3.0	—	Medium, Mn, B
$FeSO_4$† 1.0 ppm, No manganese	—	0.14	- -	Chlorosis
$FeSO_4$ 0.1 ppm, $MnCl_2$ 0.50 ppm	3.2	0.98	Heavy, Mn	Heavy, Mn
$FeSO_4$ 0.1 ppm, $MnSO_4$ 0.50 ppm	3.46	0.84	Heavy, Mn	Heavy, Mn
$FeSO_4$ 0.1 ppm, $MnCl_2$ 0.01 ppm	4.5	—	Light, B	—
$FeSO_4$ 0.1 ppm, No manganese	1.8	0.30	Medium, B	Deficiency
B. Full Hoagland macro-salt concentration. Experiment 5				
Fe-tartrate, No manganese	—	7.24	—	None
Fe-tartrate/10, No manganese	—	0.36	—	Chlorosis
No iron, No manganese	0.54	0.92	Chlorosis	Chlorosis
$FeSO_4$ 0,1 ppm, $MnCl_2$ 0.025 ppm	7.8	—	None	—

* Light, Medium, Heavy, *etc.* refer to amount of spotting. Mn and B refer to type of spotting.

† Low Mn $FeSO_4$.

from $^1/_5$ to normal or twice normal. These results are in agreement with Figures 1 and 2 and show the effect of increased salt concentration on Mn-uptake.

The effect of Fe-source and Mn-treatment on yield is shown in Table III. The data presented are for plants grown in two different experiments at different times of the year. Plants were grown in culture solutions in the presence and absence of added A4 (Cu, Mo, Zn, and B) at $^1/_5$ and full Hoagland concentrations of macro-salt. The source of iron for all experiments, unless otherwise stated was low-Mn $FeSO_4$. In the presence of A4 and Mn the yield was relatively high, with yield a function of the amount of Mn added and maximum yields obtained at 0.01 and 0.02 ppm Mn. In the absence of all micro-nutrient the yield was extremely low. In the absence of A4 the addition of Mn increases the yield slightly but the plants still exhibit deficiencies due to the absence of A4. The addition of Fe to the solutions in the form of Fe-tartrate produced yields in the absence of A5 which were the equivalent of the maximum yield obtained with $FeSO_4$ in the presence of A5. This high yield suggested that Fe-tartrate must contain a considerable number of micro-nutrient impurities. An analysis of 2 samples of Fe-tartrate showed

Mn to be present at 0.03 and 0.036%. Thus normal additions of 1 ml of 1% Fe-tartrate solution twice weekly to each litre of nutrient solution for a period of 6 weeks adds enough Mn to be equivalent to an initial Mn-concentration of 0.037 and 0.043 ppm. Both of these concentrations of Mn cause light to moderate spotting of barley leaves at $^1/_5$ Hoagland and trace spotting at full Hoagland macro-salt concentrations. Reduction of Fe-tartrate to $^1/_{10}$ of the above amount resulted in very low yields and development of Mn-deficiency symptoms. Evidently Fe-tartrate has sufficient Cu, Mo, Zn, B, and Mn to produce good barley growth when added twice weekly at the suggested concentrations.

Since the Mn had been added in all experiments as $MnCl_2$ it was possible that the symptoms were due to Cl and not to a Mn-toxicity. However the addition of $MnSO_4$ to the nutrient solution produced the same yields and the same heavy necrosis (Table III) as were obtained with $MnCl_2$ showing that the symptoms were due to Mn and not to the added Cl.

During the course of the experiments it was noted that with shorter days and lower temperatures the necrotic symptoms became weaker. Thus plants grown in the period from October 15 to November 27 showed only light spotting at 0.5 ppm Mn and moderate spotting at 5.0 ppm Mn whereas plants grown in the period from June to August showed heavy spotting at 0.5 ppm Mn with moderate spotting at 0.05 ppm Mn.

The effect of Mn and B on plant species was examined. Romaine lettuce, barley, and tomato plants were grown in Hoagland culture solutions under the following conditions: Presence and absence of micro-nutrients; presence of micro-nutrients and silica; Cu, Mo, and Zn at low levels while Mn and B were varied independently. When Mn was present in the solution at 0.5 ppm spots appeared on the oldest lettuce leaves. When B was present in the solution at 0.5 ppm the margins of the oldest leaves showed severe burn with large necrotic areas between the veins of the leaves. The addition of sodium silicate to the culture solutions prevented the appearance of the spots on the leaves but apparently had no effect on the severity of the burn. No visual toxicity symptoms were observed on tomato plants at 0.5 ppm of Mn or B. Toxicity symptoms as found on lettuce leaves are shown in the photograph in Plate II. A Mn-concentration of 0.01 ppm was observed to be deficient for both lettuce and tomato

plants and was evidenced by a marked decrease in yield. The absence of all micro-nutrients produced small chlorotic plants.

SUMMARY

Atlas barley plants grown in standard Hoagland culture solutions developed dark brown necrotic spots on the older leaves. The symptoms varied from small freckle-like spots to large blotchy areas and were found to be associated with the concentrations of Mn and B in the culture solutions. An increase in the concentration of Mn or B in the solutions increased the intensity of the spotting. A decrease in the Mn- and B-concentrations to 0.025 ppm, or one-twentieth of the normal Hoagland value, caused the spots to almost completely disappear. Mn- and B-concentrations of 0.025 ppm are optimum only under a particular set of conditions. In deciding what concentration of Mn and B to use the number of plants, volume of solution, macro-salt concentration, and season must be taken into consideration. The recommended Hoagland concentrations of Mn and B were only slightly toxic to lettuce and non-toxic to tomato plants. Barley plants grown in the winter were able to tolerate much higher concentrations of Mn and B before showing toxicity symptoms. Mn- and B-toxicity symptoms on lettuce and barley are compared in photographs.

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UNTERSUCHUNGEN ÜBER DEN EINFLUSS DES LICHTES AUF DIE P_2O_5-AUFNAHME BEI KEIMPFLANZEN

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Aus dem Institut für Agrikulturchemie und Bodenkunde der Universität Göttingen

Über die Aufnahmegeschwindigkeit der Nährstoffe durch die Wurzel und die Transportgeschwindigkeit in der Pflanze ist uns recht wenig bekannt. Da uns die Leitisotopenmethode die Möglichkeit gibt, schon kleinste Mengen schnell und exakt nachzuweisen, haben wir in einem kleinen Versuche geprüft, wie schnell die Phosphorsäure durch Keimpflanzen aufgenommen wird. Gleichzeitig wurde untersucht, ob der Aufnahmeverlauf der Phosphorsäure im Licht ein anderer ist als im Dunklen.

Auf Aptila-Keimschalen wurden je 50 Roggen- und Haferpflanzen in einer P_2O_5-freien Nährlösung aufgezogen. Als Lichtquelle dienten 8 Osram-Leuchtstoffröhren mit insgesamt 520 Watt (3000 Lux in Schalenhöhe). Nach 19 Tagen Wachstum im 12-Stunden-Tag, erhielten alle Gefässe eine Gabe von 231 mg Superphosphat (50 mg P_2O_5/ Gefäss) das mit P^{32} markiert war. Die Zugabe erfolgte bei einem Teil der Gefässe zu Beginn der Lichtperiode, bei dem anderen Teil 12 Stunden später, zu Beginn der Dunkelphase. 15, 30 Min., 1, 2, 4, 6, 8 und 12 Stunden nach der jeweiligen Zugabe des markierten Superphosphats wurden von den 4 Wiederholungen je Aptila-Schale ein Blatt geerntet, die 4 Blätter der Parallelen gemeinsam in Zellophanpapier um ein Stabzählrohr (Al-Beta-Zählrohr) gelegt und die Impulse gemessen.

Tabelle I zeigt, dass die P_2O_5-Aufnahme im Licht schneller verläuft, als im Dunklen, und dass sie bei Hafer langsamer erfolgt als bei Roggen.

Zur Kontrolle der Ergebnisse wurde der Versuch in abgewandelter

* Prof. Dr. F. Scheffer, Göttingen, Nikolausberger Weg 7.

TABELLE I

P^{32}-Aufnahme * von Roggen und Hafer (19 Tage alt) im Licht und im Dunklen

Zeit (Stunden)	1/4	1/2	1	2-	4	6	8	12
Roggen								
Hell	9	18	27	45	245	348	418	1300
Dunkel	0	2	17	14	31	35	75	196
Hafer								
Hell	0	8	14	20	145	263	190	466
Dunkel	0	4	2	16	32	71	95	280

* Impulse/Min. (Nulleffekt abgezogen)

Form wiederholt und zwar nur mit Roggen. Die Zugabe des markierten Superphosphats - dieses Mal 32 mg P_2O_5/Gefäss – erfolgte bereits am 10. Tag nach dem Ansetzen. Zur Auswertung gelangten an Stelle einzelner Blätter alle Sprosse einer Aptila-Schale. Der Versuch lief mit je 2 Wiederholungen. Die Messung erfolgte wie oben (Tabelle II*a*).

TABELLE II

P^{32}-Aufnahme von Roggen (10 Tage alt) im Licht und im Dunklen

a) *Impulse/Min. und Gefäss:*

Zeit (Stunden)		1/2	1	2	4	6	8	10	12
Hell:	Reihe a	0	22	18	268	347	671	1267	1063
	Reihe b	0	19	105	236	548	845	806	—
	Mittelwert	0	21	62	252	448	758	1036	1063
Dunkel:	Reihe a	0	18	14	64	43	327	243	338
	Reihe b	0	14	35	82	145	218	181	—
	Mittelwert	0	16	25	73	94	273	212	338

b) *Impulse/Min. bezogen auf 1 g Trockensubstanz*

Zeit (Stunden)		1/2	1	2	4	6	8	10	12
Hell:	Reihe a	0	73	74	800	1080	2070	2800	3100
	Reihe b	0	63	350	605	1470	2100	2370	—
	Mittelwert	0	68	212	703	1275	2085	2585	3100
Dunkel:	Reihe a	0	45	45	174	192	990	815	1190
	Reihe b	0	35	117	250	518	690	700	—
	Mittelwert	0	40	81	212	355	840	758	1190

Nach der Messung mit dem Aluminium-β-Zählrohr wurde die Trockenmasse der Sprosse bestimmt, diese verascht und die Asche mit verdünnter HNO_3 aufgenommen.

In einem aliquoten Teil dieser Lösung wurde im Flüssigkeitszählrohr die Messung wiederholt. Die so erhaltenen Impulse/Min., bezogen auf 1 g Trockenmasse, geben Tabelle II*b* und Abb. 1 wieder. Die Ergebnisse des ersten Versuches wurden somit bestätigt.

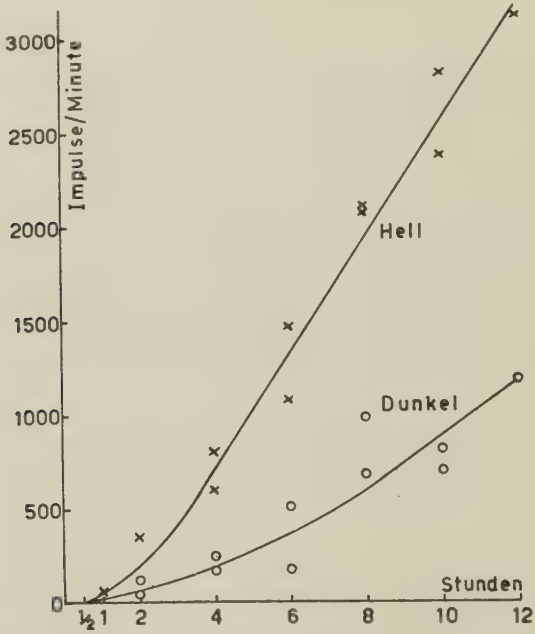

Abb. 1. $P_2^{32}O_5$-Aufnahme von Roggenkeimlingen im Licht und im Dunklen (Impulse/Minute, bezogen auf 1 g Trockenmasse). Mittelwerte aus 2 Wiederholungen (Tab. II*b*).

Es lässt sich zunächst feststellen, dass bereits nach einer halben Stunde Spuren von $P_2^{32}O_5$ in den Spross eingewandert sind. Dabei erfolgt von Anfang an eine kontinuierliche Differenzierung in die Vergleichsgruppen, die einmal unter Lichteinfluss gehalten wurden und zum anderen unter Lichtabschluss standen. Die $P_2^{32}O_5$-Aufnahme war in der Lichtphase, besonders bei Roggen, wesentlich höher als in der Dunkelphase.

Zu ähnlichen Ergebnissen kommen Schönnamsgruber [8] bei jungen Pappelpflanzen und Helder [3] beim Mais. Bei der Frage nach den Zusammenhängen zwischen Licht und P_2O_5-Aufnahme bewegen wir uns noch im Bereich der Vermutungen, die den äusserst komplizierten Vorgang der Ionenaufnahme erklären könnten [5].

Geht man der Lichtwirkung nach, so finden sich Ansatzpunkte für einen Lichteinfluss auf die Ionenaufnahme bei 2 Vorgängen:

1. Aufgrund des erhöhten Angebotes an Assimilaten steigen im Licht mit der Gesamtatmung auch die Anionenatmung und damit der aktive Ionentransport und die Ionenaufnahme.
2. Durch den lichtbedingten Prozess [6] des Anioneneinbaus (hier P_2O_5) in die organische Substanz wird ein Sättigungsdefizit der Wurzel aufrechterhalten, so dass nach einem Abtransport der Ionen weitere Ionen aufgenommen werden können. Der Abtransport muss aber durch stets bereitgestellte spezifische Trägerstoffe gesichert sein.

Beide Prozesse sind nur in Verbindung miteinander zu sehen, wobei über die Art der Verbindung keinerlei Klarheit besteht. Ob durch den Einbau unverbundener, accumulierter Anionen nur die Freistellung spezifischer Trägerstoffe bewirkt wird, der Vorgang der Aufnahme jedoch in jedem Fall Energie verbraucht, oder aber die aktive Ionenaufnahme nur bei einem Ionentransport aus niedrig konzentrierter Aussenlösung in höhere Konzentration der Zelle vorliegt [4], darüber geben die Ergebnisse keine Auskunft.

Die vorliegenden Versuchsergebnisse liessen sich so mit einer indirekten Wirkung des Lichtes auf die Nährstoffaufnahme erklären. Diese Vermutung wird allerdings durch andere Arbeiten eingeschränkt. So berichten Hanson und Biddulph [2], dass bei Bohnen nicht die Aufnahme, sondern der Aufwärtstransport von P_2O_5-Ionen im Spross im Licht zunimmt. Wenn auch dieses Ergebnis nicht schlechthin auf unsere Versuche ausgedehnt werden darf, so zeigt es doch, dass noch andere Faktoren eine Rolle spielen können. Über den Mechanismus des Ionenübertrittes in die Gefässe herrscht noch eine unklare Vorstellung. Das von Hanson und Biddulph gefundene Ergebnis liesse sich mit einer im Licht erhöhten Transpiration erklären. Durch einen verstärkten, passiven Aufwärtstransport von Ionen wird ein ähnlicher Effekt erreicht, wie durch den Einbau von Anionen in die organische Substanz, nämlich die Aufrechterhaltung eines gewissen Sättigungsdefizites. Vielleicht entspricht diese Vorstellung der von Hylmö [4], der einen passiven Ionenstransport annimmt. Nach seinen Untersuchungen läuft nämlich die Aufnahme in einem ziemlich weiten Bereich der Transpiration konform. Diese Konformität wird von Brouwer [1] nur insoweit bestätigt, als er bei Transpirationserhöhung eine zusätzliche Aufnahme, bei

Transpirationserniedrigung jedoch kein Absinken der Cl- und P-Aufnahme findet. Auf Grund dieses Befundes lehnt Brouwer einen passiven Ionentransport durch den Transpirationsstrom ab.

Wir müssen jedoch die Möglichkeit zumindest einer teilweisen Bedingtheit der erzielten Ergebnisse durch die Transpiration einräumen, wenngleich Schönnamsgruber [8] bei seinen Untersuchungen diesen Einfluss nicht feststellen konnte. Man kann vielleicht sagen, dass die Transpiration den Ionentransport beschleunigt, nicht aber allein dafür verantwortlich ist, sondern wir müssen auch die Assimilationstätigkeit der Pflanze – zu mindest für die P-Aufnahme – mitverantwortlich machen.

ZUSAMMENFASSUNG

1) 10 bzw. 20 Tage alte Roggen- und Haferkeimlinge nehmen nach einer P_2O_5-Hungerperiode P_2O_5 sehr schnell auf. Schon nach 30 Minuten lassen sich P^{32}-Atome in den Blättern nachweisen.

2) Die P_2O_5-Aufnahme ist bei Belichtung wesentlich intensiver als bei Lichtabschluss.

3) Roggenkeimlinge nehmen (besonders bei Belichtung) mehr und schneller P_2O_5 auf als Haferkeimlinge.

4) Die Bedeutung des Lichtes für die P_2O_5-Aufnahme wird in der im Licht erhöhten Assimilation gesehen, wodurch ein stärkerer Nachschub an P_2O_5 erforderlich wird.

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THE EFFECTS OF CUTTING, LIGHT INTENSITY AND NIGHT TEMPERATURE ON GROWTH AND SOLUBLE CARBOHYDRATE CONTENT OF *LOLIUM PERENNE* L. *

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INTRODUCTION

At the Central Institute of Agricultural Research (C.I.L.O.) grass growth throughout the season has been investigated for several successive years. It appears from these investigations that, after maximum growth in May, there is a reduction in growth rate with a minimum at the end of June or the beginning of July, followed by a rise in August [2]. This so-called "midsummer depression" has also been found by other workers in other countries [5 15 30] and it has been demonstrated [5 17] that it can not be eliminated by an adequate supply of water. It is supposed, therefore, to be linked with the reproductive phase of the plant, which usually occurs at the beginning of the growth depression, or with climatic influences or with both.

In order to gain a better insight into the diverse climatic factors which may influence grass growth, the effect of cutting itself on growth under controlled conditions has been investigated as well as the effects of different night temperatures and light intensities on regrowth after cutting. Since several previous investigators, see *e.g.* Weinmann [40], have mentioned the importance of carbohydrate reserves for regrowth after cutting the carbohydrate content of the plant has also been taken into consideration.

EXPERIMENTAL METHODS

The plant material used in the experiments consisted of a single clone of perennial ryegrass, *Lolium perenne* L. Whole plants were broken up into their

* Laboratory of Plant Physiological Research, Agricultural University, Wageningen Communication Nr. 151.

separate tillers. From these tillers all roots and dead leaves were removed and they were then selected for uniformity. The tillers were either planted in holes in a piece of hardboard coated with paraffin wax or in gravel in enamelled pots.

The pieces of board were of two different sizes. The smallest ones were circular with a diameter of 11 cm and had five holes each, the larger ones were rectangular, 21 × 30 cm, with 24 holes. In each hole five tillers were planted and fixed by means of two pieces of cork, also covered with paraffin wax. The smaller pieces were placed on top of one-litre glass jars covered on the outside with corrugated cardboard, the larger ones on top of rectangular glass vessels of six litres capacity, painted first black and then white on the outside. The vessels were filled with tap water until new roots had been formed. After that a Hoagland solution of half strength with the addition of micro-elements (A–Z solution) was used; iron was given as ferric ethylenediamine tetra-acetate prepared according to Jacobson [12]. The solution was not aerated as this appeared to have no beneficial effect on growth.

The enamelled pots had a capacity of approximately six litres. They were filled with gravel and planted with six groups of five tillers each. The pots were filled with tap water until new roots had been formed. After that they were flushed daily with a full strength Hoagland solution through a small opening near the bottom of the pot. All nutrient solutions were renewed once a week.

Both the glass vessels and the pots were placed in a greenhouse until the plants were ready for experimental treatments.

The experiments were performed either in the greenhouse or in a room in which light intensity and temperature could be kept constant. The light source consisted of fluorescent tubes placed as close together as possible, giving a mean light intensity of 41000 ergs/cm²/sec at the pot surface. The temperature was maintained at 20°C by fanning outside air into the room. During the summer months, however, the temperature of the outside air was often too high to keep the temperature at 20°C. By giving the plants light during the night (from 5 p.m. till 9 a.m.) the temperature was kept as low as possible but on warm days it rose temporarily to 25°C.

Before starting the experiments in the controlled room the plants were allowed to grow under the experimental conditions for at least two weeks. In all cases they remained fully vegetative.

When the plants were harvested the roots were detached; the rest of the plant was divided into two portions, viz 1) the basal part, 5 cm long, termed stubble and consisting mainly of leaf sheaths and the very short stem; 2) the remaining part, designated leaves, consisting mainly of leaf blades. From the stubble portion all dead leaf sheaths were removed. After weighing, the three fractions were dried in a forced draft oven at 70°C. When after about two hours the material was dry, it was treated at 105°C for half an hour, cooled and weighed. It was then ground and stored for analysis.

It has been shown [19 34 40] that the water-insoluble carbohydrate fractions do not show any distinct variation in respect of the factors under consideration in the present investigation. Of the water-soluble carbohydrates, the

fructosans show the greatest fluctuation. The changes in sucrose, glucose and fructose are smaller but in the same direction as the fructosans. In the present investigation, therefore only the water-soluble carbohydrates were determined, thus making possible a greater number of determinations.

The extraction of the material was carried out according to the method of De Man and De Heus [18], slightly modified by Bosman [13]. For the analyses the method of Van der Plank [28] was used.

THE EFFECT OF CUTTING

a. *Experimental*

In a preliminary experiment 14 one-litre glass jars were planted with tillers and placed in the greenhouse. Once a week the weight of the plants and the hardboard disc containing them was determined after the nutrient solution had been allowed to drip from the roots for two minutes. The number of tillers was counted once or twice weekly. The plants were divided into two groups of seven pots each and for both groups the mean values for fresh weight and number of tillers were calculated. It appeared that there were no significant differences between the two groups.

As soon as the tillers had developed into well-established plants those of one group were cut and the fresh and dry weight of the cut grass was determined. The weekly determination of growth and tiller formation was continued with the results shown in Fig. 1.

The number of tillers in the uncut plants shows a linear increase with time, whereas after cutting tillering stopped completely. There was even a slight decline in number as a few tillers died. However, five weeks after cutting the number of visible tillers rose again and during the sixth and seventh week after cutting the rate of tiller formation was even greater than with the uncut plants. Consequently, seven weeks after cutting the difference in number of tillers between uncut and cut plants was considerably reduced. Thereafter tillering continued in both groups at an almost equal rate.

The growth rate in the uncut plants did not markedly change during the experiment. With the cut plants, however, the rate was distinctly less than in the uncut ones for a period of five weeks after cutting. After that time there was an increase, and the growth rates in both groups were about equal. If one considers total production, it appears that the uncut plants produced more herbage than the cut ones. The fresh weight of the cut plants is indicated in the figure at the end of the experiment.

During the course of this preliminary experiment it appeared that the regular counting and weighing did some harm to the plants as a few pots with plants which had grown undisturbed had made much better growth. It was thought better, therefore, to examine the effect of cutting in another way, *i.e.* by taking a pot from the uncut and the cut group for analysis weekly. This would clearly increase the experimental variation and to minimize this the experiment was carried out in the temperature- and light-controlled room, with a greater number of plants per pot.

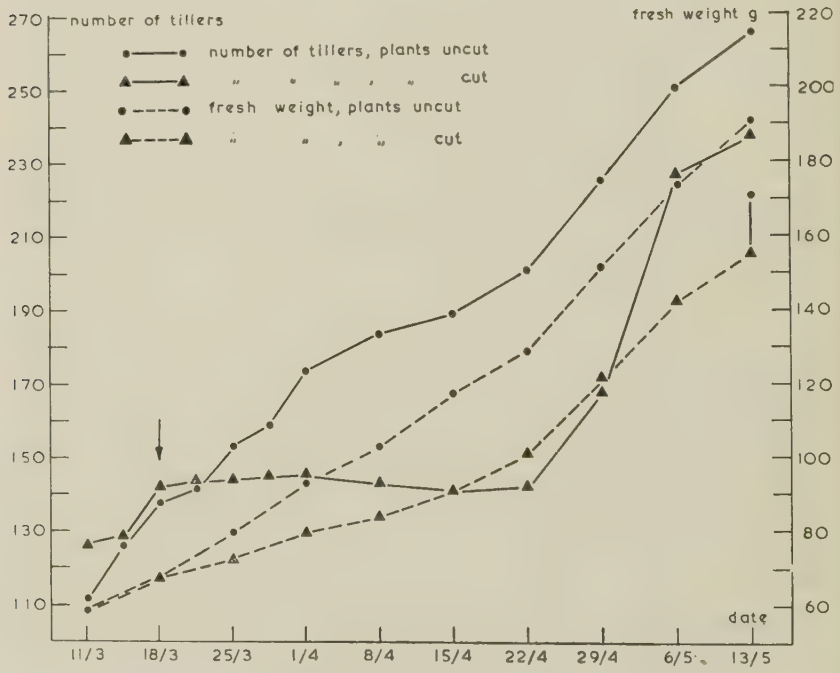

Fig. 1. The effect of cutting on fresh weight and number of tillers. The arrow indicates the time of cutting. The fresh weight of the cut material is indicated at the end of the experiment.

Ten rectangular glass vessels of six litre capacity were used, each covered with a piece of hardboard containing 24 holes. After the plants were well established they were transferred to the experimental room. Two vessels were analysed at the beginning of the experiment and of the remaining eight, four were cut. Thereafter one vessel with plants from the uncut series and one from the cut one

were analysed weekly for number of tillers, dry weight of roots, stubble and leaves and the soluble carbohydrate content of the stubble.

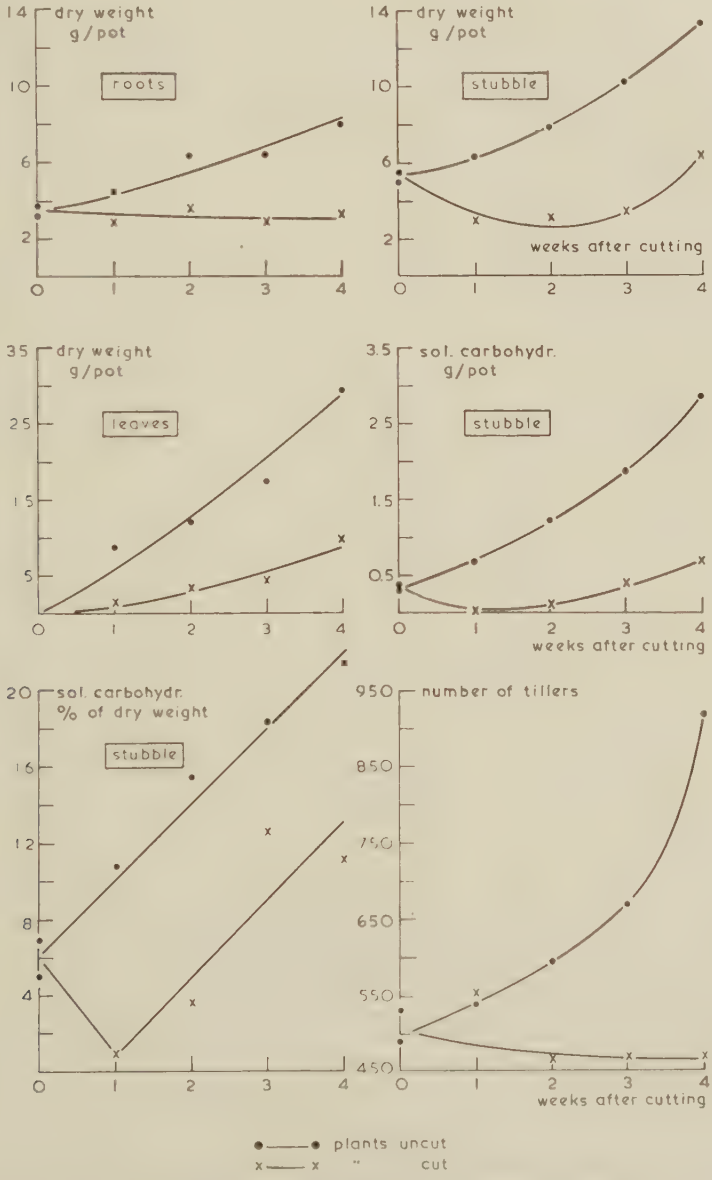

Fig. 2. The effect of cutting on number of tillers, dry matter and amount of soluble carbohydrates in the stubble. Separate graphs not on the same scale.

The results are given in Figures 2 and 3. As was expected, the number of tillers in the uncut plants increased more or less exponentially with time. In the cut series, tillering stopped completely, as in the preliminary experiment. Similarly the dry weight of roots, stubble and leaves increased exponentially with time in the uncut series. Weekly leaf growth in this series was determined by subtracting from the leaf weight at a given date the mean leaf weight of six vessels cut at the beginning of the experiment (2 used for analysis and 4 of the cut series). The values for the two vessels analysed at the beginning of the experiment have been plotted separately to give a rough idea of the individual variation.

From the results of the cut series it appears that there was no increase in root weight. The stubble weight decreased during the first half of the experiment, but then increased so that about three and a half week after cutting the original stubble weight was regained. Leaf growth only continued after cutting, but at a lower rate than in the uncut series. The dry weight increment at the end of the experiment was about one third of that in the uncut series.

The amount of soluble carbohydrates in the stubble increased exponentially in the uncut plants. After four weeks the amount was ten times higher than at the beginning of the treatment. On a percentage basis it rose from 5 to more than 20 per cent of total dry matter. In the cut plants, on the other hand, the amount dropped from about 350 to 32 mg per pot during the first week after cutting. There was then a gradual increase and after three weeks the original level was resumed. At the end of the experiment the amount was about twice as high as at the beginning. On a percentage basis it dropped from 6 to 0.5 per cent with a subsequent rise to about 12 per cent.

Both the dry weight of roots, stubble and leaves and the amount of soluble carbohydrates have been calculated per tiller and the results are given in Fig. 3. The dry weight curves of the uncut plants show that the increase in dry weight per tiller was greatest at the beginning but diminished gradually during the experiment. The root weight per tiller decreased even during the last two weeks. In the cut plants, however, there was virtually no change in root weight per tiller: the stubble weight decreased rapidly during the first half of the experiment, subsequently rising again at an increasing rate. Leaf growth rate increased gradually during the experiment with

the result that differences per tiller between the uncut and cut plants gradually diminished. Four weeks after cutting there was hardly any difference in stubble and root weight per tiller. In the rate of carbohydrate formation per tiller in the uncut plants, there was some evidence of a reduction at the end of the experiment, but the differences between cut and uncut tillers were still considerable by then, the cut ones having about half that of the uncut series.

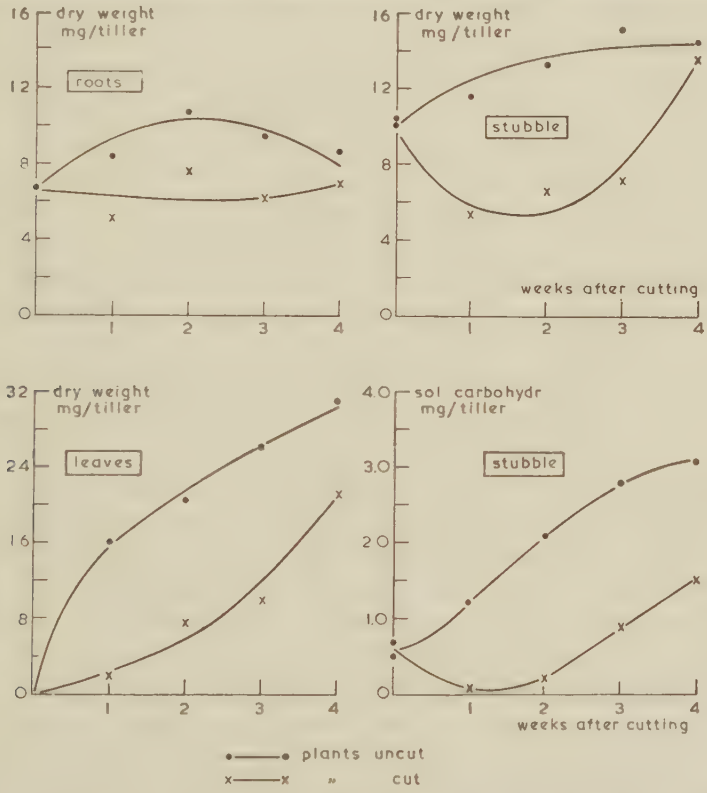

Fig. 3. The effect of cutting on the dry weight and the amount of soluble carbohydrates per tiller.

In this experiment, therefore, the principal residual effect of cutting was a lowering of the number of tillers and of the carbohydrate content per tiller.

b. *Discussion*

From numerous field experiments (see Ref. [39]) it can be concluded

that defoliation, either by mowing or by grazing, always has an adverse effect on growth expressed as dry matter production per unit time. The greater the frequency of cutting, the more is growth suppressed.

So far, however, few detailed analyses of the effect of cutting on growth under controlled conditions have been carried out. Clearly, the circumstances under which the present experiments were carried out differ considerably from those prevailing in the field. As will be demonstrated, light intensity and temperature have a strong influence on regrowth after cutting and the effect of cutting on tillering, carbohydrate content and dry matter production, as it has been found here, will certainly deviate from that under field conditions, at least quantitatively.

With plants growing undisturbed under controlled conditions both tiller formation and dry matter production went on at an increasing rate. Without doubt the rates of both processes would slow down after some time. Already the fact that, in the undefoliated plants, the rate of dry matter production per tiller was gradually decreasing during the experiment is an indication that the tillers formed were progressively less well developed, perhaps as a consequence of shading by the leaves of older tillers. Thus, at the end of the experiment, the differences between defoliated and undefoliated plants consisted mainly in a difference in the number of tillers and in the amount of carbohydrates per tiller and not in the individual tiller weight. Dibbern [8] also found almost no difference in tiller weight at the end of an experiment between uncut plants and those which were cut once or twice.

An exponential increase in number of tillers in uncut plants was also found by Mitchell [22]. But, whereas in the present experiments tillering stopped completely for at least five weeks after cutting, both at the rather high light intensity in the greenhouse and at the lower light intensity in the controlled room, Mitchell [21] only found a complete inhibition of tiller formation when the light intensity was reduced to 25 per cent of the daylight value. In his greenhouse experiments no reduction in the rate of tiller formation was observed at 14°C and only a slight retardation at 22°C as compared with undefoliated plants. This discrepancy may be due to the fact that Mitchell used young seedlings in his experiments. Connected with this is the reduction in weight per tiller in defoliated plants in

Mitchell's experiments as compared with undefoliated ones, two cuttings causing a distinctly larger reduction than one.

In the greenhouse experiment tillering in the defoliated plants was completely inhibited for five weeks. Afterwards it proceeded at an enhanced rate as compared with the undefoliated ones. Since only the visible tillers were counted it may be that bud formation continued normally during this period but that the development of these buds into visible tillers was completely or partly inhibited. Mitchell [21] also found that, under adverse conditions, it was more the development than the formation of axillary buds that was retarded.

During both experiments the plants remained fully vegetative. When they were defoliated, therefore, only the leaf blades and a part of the leaf sheaths were removed but in no case was the growing point damaged. It seems that with *Lolium perenne*, nearly removing the greater part of the photosynthetic tissue, and leaving the growing point intact, has an important influence on the mobilization and distribution of the reserve carbohydrates in the plant. Growth stopped completely except for that of the leaves which went on at a much lower rate compared with undefoliated plants. Leaf growth took place at the expense of the carbohydrates in the stubble since both the dry weight and the amount of carbohydrates in the latter decreased. However, other processes must have taken place at the same time since the diminution in stubble weight was far greater than that in the amount of carbohydrates (the separate curves of Fig. 2 are not on the same scale). After one to two weeks sufficient leaf tissue had been formed to enable the plant to restore some carbohydrates in the stubble and at about three to four weeks after cutting the original weight and carbohydrate content of the stubble had been regained. The time required to restore a certain component to its original value, will be called the recovery time for that particular component. However, it was not until five weeks after cutting that tillering was resumed.

It seemed feasible, therefore, to repeat cutting under the experimental conditions at three-weekly intervals without a reduction in the successive yields. That this is indeed the case can be demonstrated by Fig. 6 which shows that during four successive cuts at three-weekly intervals, no reduction in yield could be observed. In another experiment (unpublished) with *Dactylis glomerata* it was even possi-

ble to continue these three-weekly defoliations for about two years. Although there were considerable variations, no definite reduction in yield with time could be observed and no important changes in the number of tillers occurred.

Sullivan and Sprague [34], who also examined growth and percentage soluble carbohydrates after defoliation, obtained results which are quite comparable with the present data, not only in respect of the changes in dry weight of the different plant parts and in their carbohydrate content, but also in respect of the length of the recovery period. The drop in the percentage soluble carbohydrates in the stubble, however, was not correlated with a drop in weight.

THE EFFECT OF DIFFERENT LIGHT INTENSITIES ON THE RECOVERY AFTER CUTTING

a. *Experimental*

The experiments were carried out with material in gravel culture. The plants were cut at the beginning of the experiment. Three different light intensities were tested; the highest was 41000 ergs/cm^2/sec, the intensity used in all other experiments under controlled conditions. The second intensity was 22000 ergs/cm^2/sec and the third 12000 ergs/cm^2/sec. These two lower intensities were obtained by reducing the number of fluorescent tubes burning. On account of a shortage in space it was not possible to carry out an experiment with more than two different intensities at a time. Consequently, the high light intensity was first compared with the medium one and, in a second experiment, with the low light intensity. Since the plant material differed in vigour and initial carbohydrate content between the two experiments they can only be compared qualitatively.

The results are given in Figure 4 and Table I. From Fig. 4 it can be seen that the course of dry weight and soluble carbohydrates in the stubble at high light intensity was the same as already shown in the preceding section for the cut plants. There was a decrease both in stubble weight and in the amount of soluble carbohydrates, followed by an increase after one to two weeks so that the original status has been reached again about three weeks after cutting.

At a medium light intensity, however, there was a gradual decrease in stubble weight per pot but no distinct increase could be observed afterwards. The amount of soluble carbohydrates dropped

to a lower value than at the high light intensity; recovery was very slow but quite definite.

At a low light intensity the drop in stubble weight was steeper

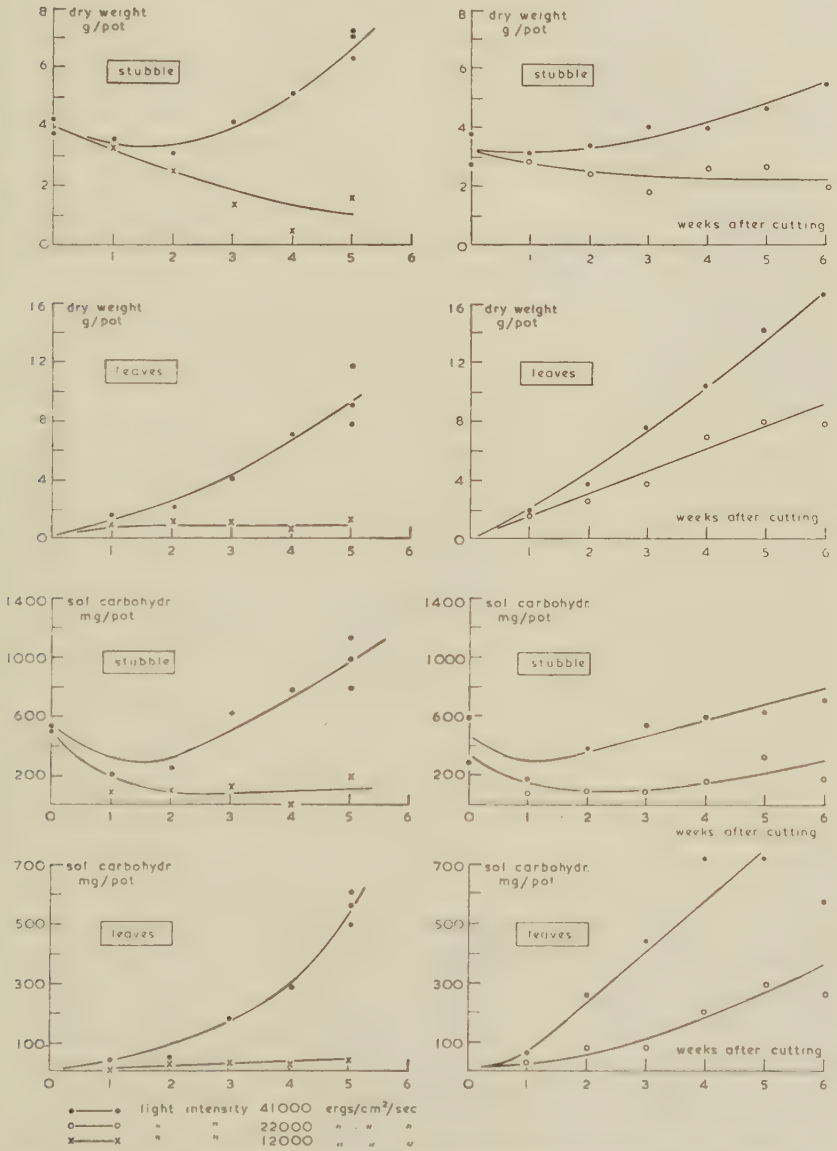

Fig. 4. The effect of different light intensities on dry weight and amount of soluble carbohydrates.

than with medium light intensity and there was no sign of recovery. The difference between a medium and a low light intensity in the drop in the amount of carbohydrates was relatively little but at the low intensity there was no distinct recovery.

The rate of leaf growth decreased with decreasing light intensity. At the medium intensity, it was reduced to about 50 per cent and at the low light intensity regrowth took place only during the first week after cutting; after that leaf growth stopped altogether. The carbohydrate figures for the leaves are more or less similar. At the medium light intensity the rate of carbohydrate production was less than 50 per cent of that at the high intensity while at the low light intensity there was almost no carbohydrate formation.

Data for the roots have been omitted since dry weight and carbohydrate content were rather irregular. However, in both experiments the values for both dry weight and amount of carbohydrates were consistently the highest at the high light intensity.

At the lowest light intensity there was a gradual decline in the number of live tillers during the experiment. Five weeks after cutting the experiment had to be stopped or there would not have been enough material for carbohydrate analysis. At that time there remained three pots at both high and low intensities. The pots at a low light intensity were combined for analysis but those grown at a high intensity were analysed separately. The separate values for these three pots are given in the figure.

At a medium light intensity also there was a gradual decline in the number of live tillers, but it was not nearly as severe as at the low intensity. At the high intensity, there were signs of an increase towards the end of the experiment. The number of tillers in the experiment with high and medium light intensity are given in Table I.

In this table the dry weight and the amount of soluble carbohydrates per tiller for both high and medium light intensity are also shown. Since both the dry weight figures and the numbers of tillers were somewhat irregular this is also the case with dry weight and amount of carbohydrates per tiller. Nevertheless it can be seen that as far as dry weight is concerned there were no distinct differences between the two intensities. The amount of soluble carbohydrates per tiller followed the same course as the amount per pot. There was a drop during the first week after cutting and a gradual increase

afterwards. However, the drop was steeper at the medium light intensity and the recovery slower. This holds good for stubble and leaves as well as for the roots. It means that the reduction in dry weight per pot at the medium light intensity relative to that at high light intensity was primarily due to the difference in the number of tillers, whereas the differences in soluble carbohydrates per pot were caused both by the difference in tiller number and by the difference in the amount per tiller.

TABLE I

Number of tillers, dry weight and amount of soluble carbohydrates per tiller at high and medium light intensities

Weeks after cutting	Number of tillers	Dry weight per tiller, mg			Soluble carbohydrates per tiller, mg		
		Roots	Stubble	Leaves	Roots	Stubble	Leaves
High light intensity, 41000 ergs/cm²/sec							
0	229	11.4	16.5	—	0.50	2.58	—
1	260	11.6	11.8	7.0	0.18	0.70	0.27
2	233	9.7	14.5	16.0	0.30	1.62	1.11
3	193	16.6	20.8	39.0	0.65	2.79	2.26
4	286	11.8	13.7	36.3	0.58	2.04	2.57
5	307	18.2	15.1	46.1	0.99	2.38	2.34
6	316	17.6	17.1	52.9	0.65	2.27	1.83
Medium light intensity, 22000 ergs/cm²/sec							
0	228	9.8	12.1	—	0.35	1.29	—
1	227	10.8	12.2	7.4	0.14	0.38	0.12
2	223	12.2	10.8	11.4	0.20	0.46	0.35
3	143	15.3	12.6	25.0	0.18	0.57	0.59
4	175	12.9	14.7	39.2	0.34	0.89	1.15
5	165	15.0	16.0	47.8	0.49	1.95	1.73
6	126	15.0	15.1	61.8	0.42	1.40	2.08

b. *Discussion*

Of the three light intensities used, the highest one was tried twice, each time with a different set of plants. Further, the conditions under this intensity were the same as those in the preceding section so that the results can be compared with those of the defoliated plants in Figure 2. As the different sets had not exactly the same weight and carbohydrate content there are quantitative differences but qualitatively dry weight and carbohydrate content of stubble and leaves follow the same course as has already been described in the preceding section.

When the light intensity is reduced this has a very distinct effect

on recovery after cutting. At the lowest light intensity no recovery is reflected in either dry weight or in the amount of soluble carbohydrates. The dry weight decreased regularly during the course of the experiment. Although the tillers were not counted it was observed that they died rapidly and this will have been the main cause of the decreasing weight, since the amount of carbohydrates dropped to a very low value during the first week after cutting and remained there throughout the experiment. An increase in leaf weight was only found during the first week after cutting, apparently at the expense of the available sugars. The same conclusions were drawn by Sullivan and Sprague[34] from experiments with plants in darkness. Apparently the new leaves formed could not synthesize enough sugars under this low light intensity to compensate for the losses in respiration.

At medium light intensity the amount of carbohydrates in stubble and roots was adequate to form sufficient leaf tissue for the compensation point to be surpassed, so that leaf growth continued and the amount of carbohydrates increased towards the end of the experiment.

These results have been confirmed by another, not yet published, experiment in which the rate of carbon dioxide uptake was investigated under different light intensities. It was found that just before cutting the compensation point lies at about 20000 ergs/cm^2/sec. This value is probably somewhat excessive since the plants were not cultivated under sterile conditions and there was undoubtedly some decomposition of dead plant material. But it can reasonably be assumed that the true compensation point will be between the medium and low light intensities used here.

An effect of light intensity on growth has been demonstrated by several other authors. Pritchett and Nelson[29] found that with bromegrass the greatest height was attained by plants under low light intensity but that these plants had thinner stems and tillered less than those in full light. This difference in length was not distinctly visible in the present experiments. Also Dibbern[8] found a very severe effect of low intensities. Plants under 5 per cent sunlight all died and only half of the bromegrass clones used survived at 14 per cent. Mitchell[21 22] showed that low light intensities reduce the number of tillers and dry matter production. Defoliation only had a marked influence on tillering when the light intensity was low. At low

temperatures light intensity had no influence on weight per tiller, at high temperatures the weight per tiller was highest at a low light intensity.

Evidence obtained so far shows that a reduction in light intensity gives rise to longer plants, a reduction in tiller number and a decrease in the amount of soluble sugars. Whether the reduction in tiller number is caused by a difference in rate of tiller formation or by the gradual dying of existing tillers depends on the experimental set up. When plants are cut at very low intensities, as in the present experiment, existing tillers will die. When, on the other hand, relatively high light intensities are compared, differences in the rate of tiller formation will arise.

THE EFFECT OF NIGHT TEMPERATURE ON THE RECOVERY AFTER CUTTING

a. *Experimental*

In two preliminary experiments it became apparent that temperature has a definite effect on regrowth and carbohydrate content after cutting.

In the first of these experiments (Expt. 1) four groups of plants on gravel culture were examined. Two of these groups were cultivated in the greenhouse, one remaining there day and night, the other being put outside from sunset till 7 a.m. The other two groups were grown outside, but one of them was brought into the greenhouse during the night. For the sake of brevity the first two groups will be called the greenhouse pots, divided into G–G: the pots kept continuously in the greenhouse, and G–O: the pots placed outside during the night. In the same way the other two groups are called the outdoor pots and are indicated as O–O and O–G respectively.

At the beginning of the experiment the plants in all pots were cut. The dry weight of the cut material was the same for all four groups as appears from Figure 5.

Three weeks afterwards the plants were cut again. At that time it appeared that there were definite differences in growth. The grass in the G–G pots was much longer than that in the O–O pots, the length of the plants of the two other groups being intermediate. These differences were not fully reflected in the dry weight of the cut grass. The G–G pots had by far the highest yield but, unexpectedly, the O–G pots had the lowest. Again, three weeks later, the differences in

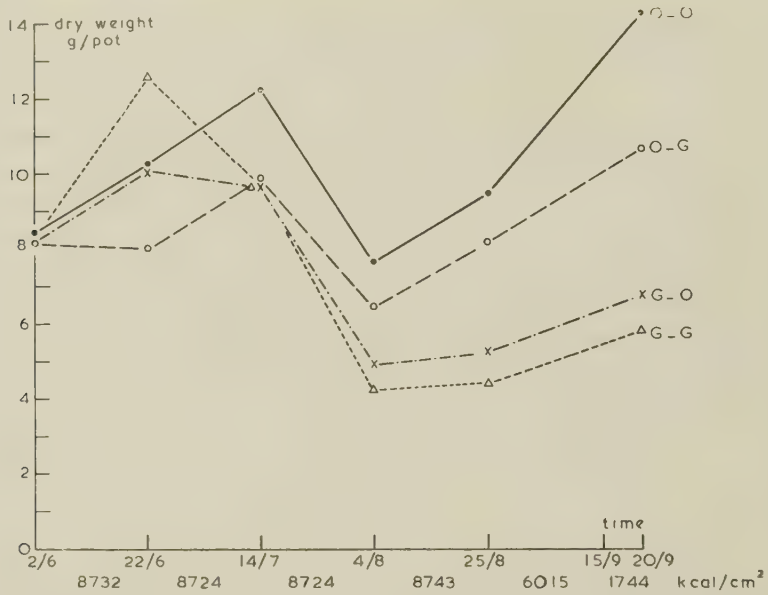

Fig. 5. The successive yields of four groups of pots under different conditions (for explanation see text).

growth habit were more pronounced. The tillers in the greenhouse pots grew erect and were very long compared with the outdoor ones. In addition it appeared that there were distinct differences in the number of tillers. This was the lowest in the greenhouse pots and the highest in the outdoor ones. As a consequence of these differences much more green tissue remained in the outdoor pots after cutting than in the greenhouse series. Although the length of the grass in the O–O pots was less than in the three other groups, the yield of the cut grass was the highest, due apparently to the much greater number of tillers. The cutting was repeated every three weeks with the exception of the last cut which took place after a four-week interval. At the third cut after the beginning of the experiment the yield in the O–O pots was the highest, next the O–G group, then the G–O group, while the G–G group had the lowest yield. This sequence was also observed in the next two cuts. After the last defoliation one pot of each group was taken for analysis. The results are given in Table II.

It appears that the differences between the four groups were not only reflected in the dry weight of the leaves but also in that of the stubble and the roots. This is caused primarily by the difference in

TABLE II

Number of tillers, dry weight and amount of soluble carbohydrates for four groups of plants (for explanation see text)

Group	G–G	G–O	O–O	O–G
Number of tillers	283	401	796	642
Roots				
Dry weight per pot. . . . . g	3.67	4.49	8.91	6.87
Soluble carbohydrates . . . . . %	3.00	3.61	3.85	3.43
Soluble carbohydrates per pot . . . . mg	110	162	343	236
Dry weight per tiller . . . . . mg	12.97	11.19	11.19	10.70
Soluble carbohydrates per tiller . . . mg	0.39	0.40	0.43	0.37
Stubble				
Dry weight per pot. . . . . g	3.34	4.77	10.78	9.27
Soluble carbohydrates . . . . . %	17.7	19.0	28.2	24.1
Soluble carbohydrates per pot . . . . mg	591	906	3040	2234
Dry weight per tiller . . . . . mg	11.80	11.89	13.55	14.44
Soluble carbohydrates per tiller . . . mg	2.09	2.26	3.82	3.48
Leaves				
Dry weight per pot. . . . . g	5.15	6.29	14.36	10.01
Soluble carbohydrates . . . . . %	8.93	6.20	11.80	7.99
Soluble carbohydrates per pot . . . . mg	460	390	1694	800
Dry weight per tiller . . . . . mg	18.20	15.68	18.04	15.59
Soluble carbohydrates per tiller . . . mg	1.63	0.97	2.13	1.25
Soluble carbohydrates total per pot. . g	1.161	1.458	5.077	3.270

the number of tillers since there was hardly any difference in the dry weight per tiller. There was also a considerable difference in the amount of soluble carbohydrates per pot especially in the stubble, the O–O pots having produced about five times as much carbohydrates as the G–G pots. However, on a percentage basis the differences were much less and, although the O–O pots had the highest percentage in all cases, the sequence differs. The G–G pots, for instance, had a higher percentage in the leaves than the G–O pots and even the O–G pots. The values of the soluble carbohydrate content per tiller are not always consistent with the total amount of carbohydrates. Only in the stubble is the sequence the same for dry weight per pot, soluble carbohydrates per pot, per cent carbohydrates and soluble carbohydrates per tiller. This, however, does not hold good for leaves and roots. But generally it can be said that there is a definite correlation between the yield in dry weight of roots, stubble and leaves per pot and the amount of soluble carbohydrates in these parts of the plant.

The second experiment (Expt. 2) was carried out under controlled conditions. Four series of pots with plants in gravel cultures were placed in the controlled room during the day (16 h, temperature 20°C, light intensity 41000 ergs cm^2 sec). During the night these groups were placed in darkrooms at different temperatures, viz. 3°C, 10°C, 20°C and 28°C. In order to obtain fairly rapid adjustment in root temperature, the two groups having the lower night temperatures were flushed with a nutrient solution at 10°C at the end of the day period and with a solution at 20°C at the end of the night period; the other two groups were always flushed with a solution at 20°C. By giving different temperatures during the night only, any effects of temperature on photosynthesis could be eliminated.

The results were similar to those obtained in the greenhouse–outdoor experiment. Leaf elongation was greatest at 20°C and lowest at 3°C. Three weeks after the beginning of the experiment the plants were again cut. The plants at 20° gave the highest yield, those at 3° the lowest (Fig. 6).

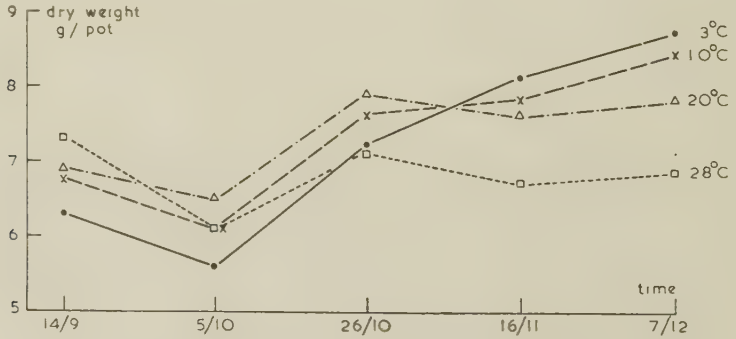

Fig. 6. The successive yields of four groups of pots at different night temperatures.

Three weeks later the sequence was 20° > 10° > 3° > 28°. After another period of three weeks it was 3° > 10° > 20° > 28°. By this time there were considerable differences in the number of tillers; at the highest temperature many tillers had died whereas at the lowest temperature no dying of tillers was found. A further three weeks later, at the end of the experiment, the same sequence was found. The pots were now put under an 8 hour day and a 16 hour night, the differences in night temperature remaining the same. It appeared that after three weeks the yields were much lower and that the 28°

plants were nearly all dead. One pot of each series was analysed for carbohydrate content in the stubble, giving the following data: 3°C, 9.6%; 10°C, 4.3%; 20°C, 1.7%. Of the 28°C pots there was not enough material available for an analysis. At subsequent cuts the other series also deteriorated, first the 20° pots, then the 10° pots and finally the 3° pots.

From both experiments it appeared that night temperature has a definite influence on grass growth but that this influence is rather complicated. If one considers dry matter production under different night temperatures, the ultimate result was the same in both experiments, viz that the plants at the lowest temperature had the highest production of dry matter and that these differences were correlated with differences in the amount of carbohydrates.

In trying to obtain further information on this effect of night temperature a more detailed experiment was carried out (Expt. 3). Sixteen pots with plants in gravel culture, cultivated in the greenhouse in the usual manner, were defoliated and divided into two groups. Both groups received a pretreatment at different night temperatures for three weeks. One group had a night temperature of 3°C for eight hours, the other group of 28°C. The conditions during the day were the same for both groups, viz 20°C and a light intensity of 41000 ergs cm^2 sec. At the end of the pretreatment all the pots were cut again and one pot of each group was analysed for number of tillers, dry weight and soluble carbohydrate content of roots and stubble. The remaining pots were held under the same conditions as they had before. Every week one pot of each group was analysed. The results of the experiment are given in Figure 7.

At the beginning of the experiment there was not much difference between the dry weight of the cut leaves in each group. The pots at a 3° night temperature had a mean leaf weight of 27.5 g per pot and those at 28° of 24.3 g per pot. Furthermore there was hardly any difference in the dry weight of roots and stubble between the first two pots analysed. Although there were rather big individual variations it can be seen that the differences between the two series became gradually more pronounced. In all cases the dry weight of roots, stubble and newly produced leaves was the highest at the low night temperature. The same can be said of the amount of soluble carbohydrates. With both temperatures there was a drop in stubble weight and the amount of soluble carbohydrates after cutting as has

been found in other experiments. At the high night temperature, however, there was no distinct recovery.

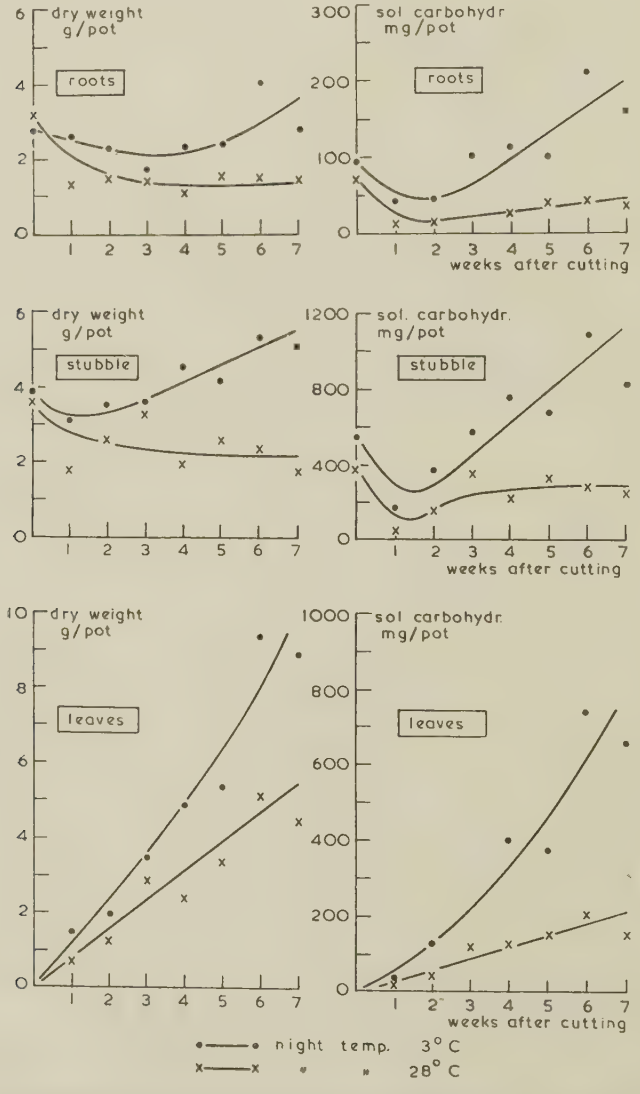

Fig. 7. The effect of two different night temperatures on dry weight and amount of soluble carbohydrates.

The number of tillers and the calculated dry weight and carbo-

hydrate content per tiller for roots, stubble and leaves at both night temperatures are given in Table III.

TABLE III

Number of tillers, dry weight and amount of soluble carbohydrates per tiller at two different night temperatures

Weeks after cutting	Number of tillers	Dry weight per tiller, mg			Soluble carbohydrates per tiller, mg		
		Roots	Stubble	Leaves	Roots	Stubble	Leaves
Low night temperature, 3°C							
0	304	8.9	13.1	—	0.32	1.83	—
1	304	8.6	10.3	4.9	0.13	0.64	0.16
2	278	8.2	12.7	7.2	0.17	1.39	0.49
3	270	6.4	13.2	13.1	0.38	2.17	—
4	302	7.6	14.9	16.3	0.37	2.54	1.34
5	324	7.4	13.3	16.7	0.31	2.13	1.18
6	406	9.9	13.2	23.2	0.53	2.72	1.84
7	484	5.9	10.6	18.3	0.34	1.75	1.36
High night temperature, 28°C							
0	316	10.1	12.0	—	0.23	1.23	—
1	232	5.7	8.1	3.3	0.04	0.25	0.05
2	203	7.6	13.3	6.4	0.06	0.87	0.18
3	267	5.4	12.5	8.6	—	1.36	0.45
4	157	6.6	12.8	15.5	0.16	1.43	0.81
5	244	6.5	10.9	13.9	0.17	1.39	0.68
6	229	6.6	10.6	22.4	0.18	1.32	0.97
7	190	7.3	9.4	23.4	0.18	1.39	0.80

Although there were large variations between the individual pots as to the number of tillers and the dry weight and carbohydrate content of the different plant parts, the following conclusions seem to be warranted:

1. At 3° night temperature tiller formation was resumed about five weeks after cutting; at 28° no increase in tiller number was found during the experiment.

2. There is no distinct difference in the dry weight per tiller between the two temperatures but a very large difference in the amount of soluble carbohydrates per tiller.

3. In roots and stubble there is no very pronounced drop after cutting in the dry weight per tiller, and, consequently a recovery time can not be established. In the amount of carbohydrates, however, the drop is considerable. For the stubble the recovery time is about three weeks at both temperatures; for the roots this time is

also about three weeks at 3°C, whereas no complete recovery was found at 28°C.

b. *Discussion*

The effect of night temperature on growth can be divided into two parts, viz a short-term effect within the first three-weekly growth period and the effect after repeated cutting.

1. The short-term effect

In Experiment 1 the four series can be combined two by two. The G–G and G–O pots had the same conditions during the day but differed in their night temperature. The temperature in the greenhouse was about 3°C higher than outside during the first three weeks of the experiment. The same holds good for the O–O and O–G pots. In comparing the dry weight of the leaves at the first cut it appears that with the greenhouse pots the highest yield was found at the highest night temperature, whereas with the outdoor pots the highest yield was found at the lowest night temperature.

This discrepancy can perhaps be explained by differences in tiller number. Although the tillers were not counted at the time of the first defoliation, an increase had certainly taken place in the O–O pots, so that the tiller number was greater than in the O–G pots. However, the effect of different day and night temperatures on tiller number will have to be studied more thoroughly before more definite conclusions can be drawn.

Peterson and Loomis [27] found a higher yield with plants growing in a greenhouse than with plants in the open. This may have been caused by differences in temperature as well as in light intensity.

In Experiment 2 the highest yield was found at a night temperature of 20°C. At 28° the plants were the longest but some tillers had died under these conditions so that the dry weight was smaller.

Brown [4] found that different grass species have a different response to temperature, *Cynodon* having a much higher optimum than *Dactylis* or *Poa*. The same was found by Dibbern [8], who examined the effect of different soil temperatures on the growth of clones of *Bromus inermis* originating from different latitudes. The clones from southern regions had a higher temperature optimum than those from northern regions. Perennial ryegrass, being a species from temperate climate, has about the same optimum as *Dactylis* examined by Brown.

2. The effect after repeated cutting

When, in our experiments, cutting was repeated several times the highest final yield was constantly found with the lowest night temperature. The same feature has been observed by other workers.

Peterson and Loomis [27] found that the difference in yield between plants in the greenhouse and the open became less after the plants were cut. Lovvorn [16] stated that frequent cutting gave a greater reduction in yield at high than at low temperature. Dibbern [8] found with uncut clones an optimum yield at 26°C. When the same clones were defoliated, however, the highest yield was invariably found at the lowest soil temperature. Mitchell [22] also compared the yield of defoliated and undefoliated plants at two different temperatures. The undefoliated plants had the highest yield at the highest temperature, viz 22°C. When the plants were defoliated twice the yield was highest at 14°C.

It seems that time has no effect on the influence of night temperature on carbohydrate content.

Peterson and Loomis [27] have found that the sugar content was always higher in the out of door growing plants than in those growing in the greenhouse although the latter plants gave a higher yield. Likewise Brown [4] found that the content of N-free components was inversely correlated with temperature, except in *Cynodon dactylon*, which grass was also an exception in that it had a temperature optimum for growth at or above 38°C.

Unfortunately in Experiments 1 and 2 an analysis of the amount of soluble carbohydrates was only made at the end of the experiment when several defoliations had already taken place. Only in Experiment 3 was an analysis carried out three weeks after the beginning of the treatment with different night temperatures. At that time the two extreme night temperatures had not resulted in differences in yield. Nevertheless there was a difference in the amount of carbohydrates, that at 3° being distinctly higher than that at 28°. Further, during the subsequent course of this experiment the differences in carbohydrate content became much more pronounced than the differences in weight. The same conclusion can be drawn from the two other experiments. In Experiment 1, for instance, the ratio low temperature/high temperature was for stubble weight 3.23 and for the amount of carbohydrates 5.14. For the leaves these figures were respectively 2.79 and 3.68. Although there is as yet no conclu-

sive evidence, it seems that for grasses indigenous to temperate regions there is an inverse relationship between soluble carbohydrates and temperature within the range between 10° and 30°C.

It appears from Experiment 3 that the soluble carbohydrate content dropped immediately after cutting at both night temperatures but that this initial drop was much more pronounced at 28° than at 3°. As there was considerable variation between the plants of different experiments and as the plants in this experiment received a pretreatment it is not possible to compare these results quantitatively with those in the preceding sections (p. 203 and 209) in which the effect of cutting on the carbohydrate content at a night temperature of 20° was studied under otherwise similar conditions. At 28° the amount of carbohydrates in the stubble seven weeks after cutting was still below that just before cutting, whereas at 3° the original amount has already been reached again after three weeks. The same holds good for the roots. In the leaves the amount of carbohydrates at 3° was more than three times higher than at 28° at the end of the experiment. Thus, it appears that at high night temperature, just as at a low light intensity, recovery after cutting is incomplete. There was little difference in recovery time between 3° and 20° night temperature.

More or less the same results were found by Sullivan and Sprague[35] who examined the change in the percentage of different soluble carbohydrates after cutting over a period of about six weeks. That there was no recovery possible in their experiments even at the lowest temperature range used, is probably caused by the low light intensity adopted.

Comparing the results obtained in the present investigation with those of other workers it seems possible to give a more detailed picture of the influence of night temperature on grass growth. In doing this it is perhaps the best to start with Experiment 2.

At the beginning of this experiment all four groups were more or less alike as to amount of tissue per pot and, presumably, carbohydrate content. It has been shown by several workers that there is an optimum temperature for growth, lying for grasses from temperate regions round 20°C. In Experiment 2 also, when the plants were cut three weeks after the beginning of the experiment, the highest yield was found at 20°C. Not only was more dry matter produced at 20° than at other temperatures, but, also, respiration during the

night was rather high; both factors would tend to lower the carbohydrate reserves as compared with lower temperatures. On the other hand this larger amount of green leaf tissue enabled the plants to form carbohydrates at a higher rate than at either higher or lower temperatures. It seems that the net effect of these two opposing tendencies results in a lower soluble carbohydrate content compared with plants at lower night temperatures. Thus, at the first cut after the beginning of the treatment, the plants at the lowest night temperature have the highest carbohydrate content in the remaining part of the plant, although not the highest yield. Similar phenomena have been observed with other plants. Two examples may be given here. Verkerk [37] observed in the tomato an increase in growth rate but a decrease in fruit weight with increasing temperature. Decker [7] found a decrease in the ratio of photosynthesis to respiration with increasing temperature in pine seedlings. It may be that all these phenomena are caused by a lower temperature optimum for photosynthesis than for respiration [4].

At subsequent defoliations these differences in carbohydrate content increase gradually and as a result, there arise differences in the number of tillers. At comparatively low light intensities (as in the controlled room), plants at a high night temperature show a reduction in the number of living tillers, whereas those at a low night temperature keep their number constant or even show an increase. This was the case in the Experiments 2 and 3. At higher light intensities, as in Experiment 1, the number of tillers at low temperature increases much more rapidly than that at a higher night temperature. The effect of different night temperatures ultimately results in a decreasing number of tillers and in a decreasing carbohydrate content per tiller with increasing temperature. Therefore, although the rate of elongation of the leaves remains optimal at temperatures around 20°, the highest yield is always found at the lowest night temperature (between 10° and 30°C) on account of the greater tiller number.

Besides these differences in number of tillers differences in growth habit may also arise. The plants growing at a high light intensity and a comparatively low temperature tend to grow more prostrate. Compared with plants at low light intensity and higher temperatures they have more green tissue left after defoliation. This effect may enhance the differences in carbohydrate content.

GENERAL DISCUSSION

As has already been pointed out, cutting has an adverse effect on dry matter production. In the numerous field experiments on this subject it appears that the effect of cutting has usually been investigated in an established sward for a comparatively short period, at the utmost for one growing season. It can reasonably be expected, however, that undisturbed grass growth, apart from the effect on palatability and feeding value, will lead after several seasons to something that is not at all comparable to a normal pasture, in other words, that defoliation is necessary to maintain the grassland at the required quality throughout.

On the other hand too close and too frequent defoliation will also lead to deterioration in the grassland, and good pasture management lies between these two extremes.

From the results obtained in the present investigation it can be seen that the soluble carbohydrate content in those parts of the plant which remain after cutting determines more or less the growth potential. Furthermore, it has been shown that conditions, optimal for growth *per se*, often give rise to a depletion of the carbohydrate reserves after repeated defoliation. In several experiments the recovery time for the amount of carbohydrates was about three weeks and by cutting at three-weekly intervals the successive yields did not become smaller. It seems warranted to say that the plants, once they are in good condition, can be kept in this condition provided that the time between two successive cuts is not shorter than the recovery time for the amount of carbohydrates.

Although a night temperature of 20°C was optimal for growth, it may lead to a decrease of the carbohydrate reserve in a three-weekly cutting scheme. At a night temperature of 3°C, however, growth was less at first but became better than at 20°C after several cuts, apparently because, after cutting, the carbohydrate content could rise to a higher level than at 20°C before the next cut was made. It is clear that the picture is not complete because different day temperatures have not been tested. It has been shown by Went [41] that, in most cases, growth is optimal when the night temperature is several degrees lower than the day temperature. Juhren *et al.* [14] have demonstrated that this also holds good for the undisturbed growth of several grass species. Unfortunately, in connection with the problem

under consideration, no carbohydrate determinations were carried out, nor were the plants defoliated. Therefore, it has yet to be established what combination of day and night temperatures is optimal for the total yield after repeated defoliation.

As far as the results of this and other investigations show there is a positive correlation between light intensity and dry matter production [8 22 29]. The effect of high temperature can partly be compensated by a high light intensity. Thus far the effect of light intensity is far less complicated than that of temperature. However, the results given in Figure 5 seem to contradict this since there was a distinct increase in yield in all four groups with the successive cuts from the beginning of August till the end of September. In the same period the solar energy outside decreased from 8742 kcal/cm^2 in the first intercutting period to 6015 kcal cm^2 during the first three weeks of the second. It must be realised, however, that the temperature decreased at the same time and this may have had a greater influence than the decrease in solar energy. It remains possible, furthermore, that there is also an effect of the length of day as has been claimed by several authors [5 9 16 27].

Under conditions which promote fairly rapid recovery in carbohydrate content of the stubble, such as high light intensity at a rather low temperature, tillering is also resumed relatively early. Thus, in the outside pots in Experiment 1 (see p. 215), there was an increase in the number of tillers during the experiment, which means that tillering was resumed within three weeks of cutting. On the other hand, under less favourable conditions, the number of tillers remains constant or may even decrease as in the experiment with low light intensity. Similar results were found by Harrison [11]. With *Poa pratensis* new rhizomes were formed at low temperature, whereas at a high temperature many rhizomes died.

It has also been found [4 6 11] that root growth only takes place under conditions favourable to carbohydrate synthesis. However, no distinct differences in root weight relative to the weight of stubble and leaves could be found in the present experiments.

In an attempt to complete the picture it can be stated that in the grass plant the effect of climatic factors on growth must be studied in relation to cutting, since this gives a picture quite different from that shown by uncut plants. Factors, such as optimal temperature or a high nitrogen fertilization [10 11 23 24 32 36], which tend to promote

leaf growth, tend to lower the soluble carbohydrate content after repeated cutting and, as a consequence, tillering and root growth come to a standstill and the successive cuts may decrease in weight. On the other hand factors which tend to inhibit leaf growth or which tend to increase apparent photosynthesis (*e.g.* high light intensity), tend to increase the carbohydrate reserves. Together with this rise in carbohydrate content, and perhaps connected with it, goes an increase in tiller number and the formation of new roots. Due mainly to this increase in tiller number, the successive cuts tend to increase in weight.

Turning now to grass growth under normal field conditions it can be shown that during the winter the carbohydrate content is normally high [1 5 19 31]. In early spring the formation of new roots starts before any leaf growth is visible [5 13 19 33] and, as the rise in temperature proceeds, conditions become increasingly favourable and the upper parts of the plant start growing. During spring and early summer, when leaf growth is very rapid, the carbohydrate content falls to much lower values. It was observed [1] that this fall takes place at the same time as stem elongation and ear formation. It may be suggested that the high yields in spring are made possible by the carbohydrate content present and the favourable conditions for growth and that, thereby, the major part of these reserves is exhausted. This is even enhanced by stem elongation and ear formation as is suggested by several authors [1 19 25 38].

It can reasonably be stated, therefore, that both vigorous growth and heading lower the carbohydrate reserves and, in consequence, cause the observed fall in subsequent yields, usually called the midsummer depression. Perhaps, also, the higher night temperatures at this time of the year have an adverse effect on the recovery of the carbohydrate reserves. After this steep drop the carbohydrate content rises gradually during August and September and is accompanied by a rise in grass yield. It cannot yet be decided which climatic factors are responsible for the gradual increase in carbohydrate content over this period, since temperature and day length effects have not been fully investigated both separately and in combination. When the temperature becomes too low in the autumn, grass growth decreases rapidly, but there is, at the same time, a steeper rise in the carbohydrate content of the stubble and a second period of root formation.

Thus, the evidence so far indicates that under field conditions also,

factors which tend to promote leaf growth tend to lower the carbohydrate reserves in the plant and to inhibit other growth processes such as root growth and tiller formation. When conditions later become unfavourable for leaf growth, the carbohydrate content rises and root growth and tillering can be resumed until the temperature becomes so low that growth stops altogether.

It must be mentioned, however, that with uncut grass results have been found which show a more or less opposite trend in soluble carbohydrate content, viz a rise in early summer and a fall in the autumn [20 26 38]. This trend has been found particularly in the herbage; McIlvanie [20] showed that for the roots the curve of carbohydrate content was more like the U-form found in the stubble of the regularly cut grass. Norman and Richardson [26] found that the maximum in fructosan content in the herbage was reached as early as the end of May. In later work Norman [24] found a U-shaped curve with undefoliated cocksfoot plants, the fructosan content being the lowest in the middle of June. According to McIlvanie [20] and Waite and Boyd [38] the maximum in sugar content coincides with the time of flowering and seed formation.

These differences in trend of the soluble carbohydrate reserves throughout the season are undoubtedly connected with differences in management. That this is indeed the case has been demonstrated by Waite and Boyd in a second investigation [39] in which regularly cut grass was studied under the same conditions as the uncut grass described in their first publication. In the second case, instead of a maximum, a minimum was found in the summer.

Although it has often been stated that in elongation and flower formation the plant has to rely, at least partly, on the carbohydrate reserves, the uncut plant seems to be able to increase the sugar content until after flowering. The subsequent fall is undoubtedly connected with the gradual dying of the herbage. Clearly these results demonstrate the great influence of grassland management on the soluble carbohydrate content.

Although in this article a general indication has been given as to the influence of some climatic factors on grass growth and carbohydrate content, much further work is required, both in the laboratory and under field conditions, before the interrelations between climatic factors, stage of development, fertilization and management will be fully understood.

SUMMARY

The effect of cutting vegetative plants of a single clone of perennial ryegrass was investigated and the influence of night temperature and light intensities on growth and carbohydrate content studied both in a greenhouse and in a temperature- and light-controlled room. The results can be summarized as follows:

1. Immediately after cutting tiller formation stops; leaf growth continues, but at a much lower rate than with uncut plants under the same conditions. The soluble carbohydrate reserves in stubble and roots are partly consumed in the formation of new leaves. Consequently the weight of these plant parts decreases temporarily.

2. The recovery time – *i.e.* the time in which a component regains the value it had just before cutting – is dependent on light intensity and temperature. It increases with decreasing light intensity and increasing night temperature. Under the conditions normally prevailing in the artificially lighted room (light intensity 41000 ergs/cm^2/sec, temperature 20°C) the recovery time both for the amount of soluble carbohydrates in stubble and roots and for the dry weight of these plant parts, is about three weeks. The time during which there is no tiller formation is longer than the recovery time for either dry weight or sugar content, but is also dependent on temperature and light intensity. It has been observed that once tillering recommences, it proceeds at an enhanced rate compared with uncut plants.

3. With plants of similar dry weight and carbohydrate content, dry matter production is optimal at a night temperature of about 20°C. The carbohydrate content, however, was found to be inversely related to night temperature. Consequently, after repeated defoliation the highest yield is invariably found at the lowest night temperature.

4. Differences in dry matter production and in the amount of soluble carbohydrates in the different plant parts, whether they arise as a consequence of differences in cutting, temperature or light intensity, are caused mainly by a difference in tiller number and in the amount of carbohydrates per tiller, and not by differences in tiller weight.

These results are discussed in relation to the outcome of other investigations and to variations in yield under field conditions.

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FERTILIZER PLACEMENT EXPERIMENTS

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1. INTRODUCTION

Placed fertilizer is localized in bands by special distributing machines at some definite position in the soil close to the seed or seed potatoes. In some countries this method has been a common practice for a good many years, because the combination of sowing and fertilizing reduces labour costs. For example, in the United States, placement has been carried out extensively, particularly for crops grown in widely spaced rows such as maize, tobacco, cotton and potatoes. It was soon found that better yields could be obtained with placed compared with broadcast fertilizer, giving the additional advantage of saving fertilizer. During World War II placement was therefore introduced into England to restrict the use of fertilizers.

In the Netherlands the usual way of applying fertilizers is to broadcast them evenly over the surface after ploughing and before the seedbed is prepared. The fertilizers are mixed with the soil by cultivating of harrowing, so that they are localized in the upper layer of the soil (Roemer [14]). With this traditional method the plant roots do not make sufficient contact with the fertilizer particles, especially during dry periods. It is essential to apply the fertilizer below the surface into moist soil; a correct distribution in a vertical direction is therefore more important than in a horizontal direction (Torstensson [16]). Moreover, fertilizer supplements such as phosphate and potash quickly become fixed when mixed thoroughly throughout the soil and, consequently, there is a rather small uptake of nutrients when broadcast. As has been found elsewhere (Cooke [3]) our investigations show that placement gives marked advantages.

The present paper describes the results of field trials in the Netherlands. Goedewaagen [6] has already demonstrated a stimulation of branching and development of plant roots around placed fertilizer, often resulting in a total increase in the root system. De Wit [18] has recently developed a theory concerning fertilizer placement, based on experimental data, so as to gain an insight into the underlying principles; in this article his theories are tested against our results.

2. METHODS

The investigations have been carried out for several seasons on different kinds of crops and types of soil by our Institute, partly assisted by the Agricultural Advisory Service. A large number of the experiments deals with the comparison between placement and broadcasting of phosphate with oats, maize, pulse crops, potatoes and beets on soils varying in phosphate content and in phosphate fixation. The investigation with potash has been confined to potash-fixing river clay soils for spring wheat and potatoes. In addition, placement experiments with nitrogen have been tried on oats, potatoes and beets.

In most of the experiments the two methods of application were tested at three or four rates of fertilizer, in addition to controls. The fertilizers used were superphosphate, sulphate of potash, and nitro-chalk.

Broadcast fertilizer was applied to the seedbed and harrowed in shallowly; placed fertilizer was localized by hand in grooves at a certain distance from the seed (tuber). Later the experiments of our Institute were carried out by a special machine, suitable for experimental work in combination with seed sowing.

This machine, built after English design [1], consists of a Saxonia sowing machine with 2–4 seed coulters (row distance 25–67 cm), mounted on a self-propelled 4-wheeled chassis. The engine is fitted behind the tractor driver. In front, two fertilizer cylinders are erected, each delivering to two coulters (Fig. 1). The cylindrical feeding units, working on a positive-displacement top-delivery principle, place a definite quantity of fertilizer in any desired position relative to the seed. For cereals, bands were placed 2 cm and for other crops, 5–7 cm to the side of the seed. In all cases the fertilizer was applied about 2 cm below the level of the seed, for crops grown in closely spaced rows (cereals and pulse crops) in a single band on only one side of the row (a single band is preferable from a practical standpoint), for crops in widely spaced rows

(maize, potatoes and beets), in two bands on both sides of the row. The width of the band was 3–5 cm. Only granular fertilizers are suitable for accurate placement; powdered fertilizers become compacted partly through jolting and vibration from the machine and partly through the upward pressure of the piston and cause blockages in the freed tubes and coulters. It is possible to apply 48 different quantities of fertilizer by means of a gear box; the quantity from each tube is adjusted by scrapers. The machine works satisfactorily provided certain precautions are taken [1]. The variations in delivery rates are calculated by weighing the fertilizer delivered into collecting boxes

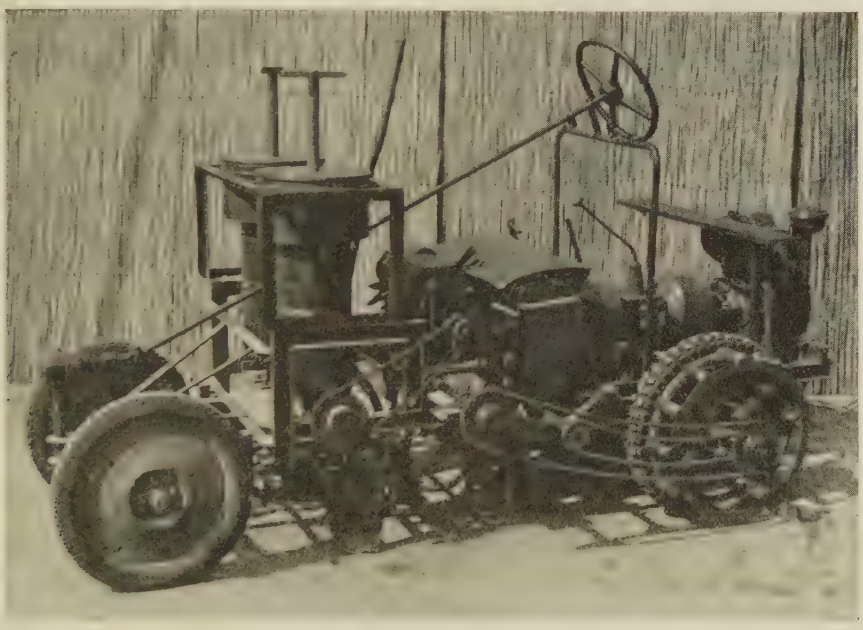

Fig. 1. Experimental fertilizer placement drill.

while travelling over a measured distance before and after the application of the fertilizer for each treatment. The coefficient of variation was 2.7%. In combination with the application of the fertilizer, the crop is sown in rows. Potatoes are afterwards hand-planted midway between the two fertilizer bands.

3. EXPERIMENTAL RESULTS

Since more than 100 experiments were carried out during 1948–1955 to compare placement with broadcasting, a detailed discussion of the results of all the experiments would be impracticable. We have

therefore tried to establish for each field trial the difference between the effect of the two methods of application on the yield in terms of a comparative value. Before discussing these comparative figures, we shall give the results of some experiments to show the significance of the effect of placement.

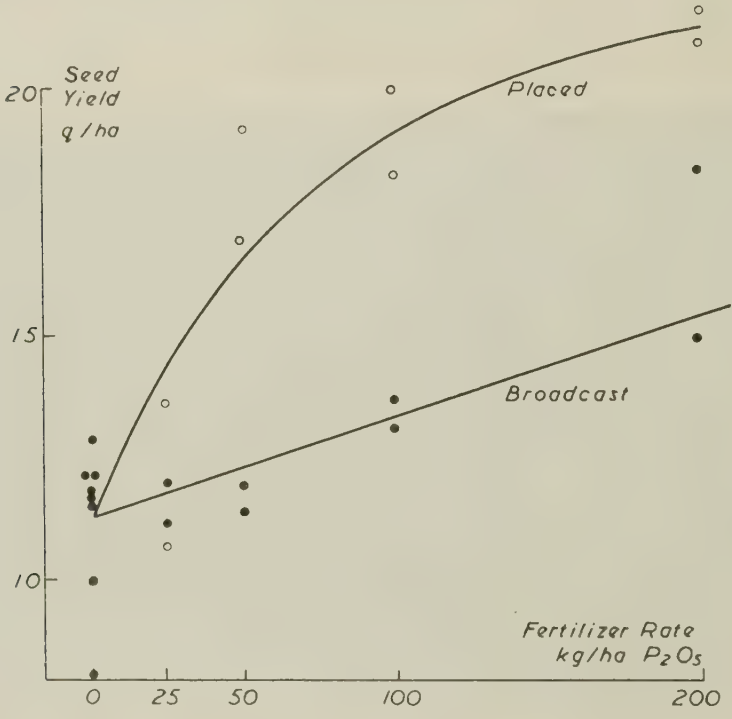

Fig. 2. Comparison between placement (○) and broadcasting (•) with phophate for beans on sandy soil (Exp. Pr 1146).

A very spectacular advantage of placement was found on soils deficient in phopshate, especially with pulse crops, maize and cereals For beans this is illustrated in Fig. 2, in which yields are plotted against the quantities of phosphate applied by placement and by broadcasting. Maize was markedly stimulated by placement of superphosphate; the crop grow more quickly, ripening was promoted and higher yields were obtained than with the broadcast dressing (Fig. 3). Fig. 4 presents the results of another of our experiments with maize. This trial was carried out to compare placement with broadcasting according to the phosphate content (P-citr value=

phosphoric acid soluble in 1% citric acid) of the soil. As a result of different dressings in previous years widely divergent phosphate contents were found. On the basis of these different phosphate conditions, both methods of application are compared at 30, 70 and 150 kg P_2O_5 as superphosphate per ha; there were also a number of control plots. The yield is considerably increased by phosphate dressing. A small quantity of merely 30 kg P_2O_5 per ha increases the yield even as much as a difference of 6 units P-citr on non-fertilized soil (6 units correspond with *ca* 260 kg. P_2O_5 per ha). The effect of phosphate placed in bands is even considerably better.

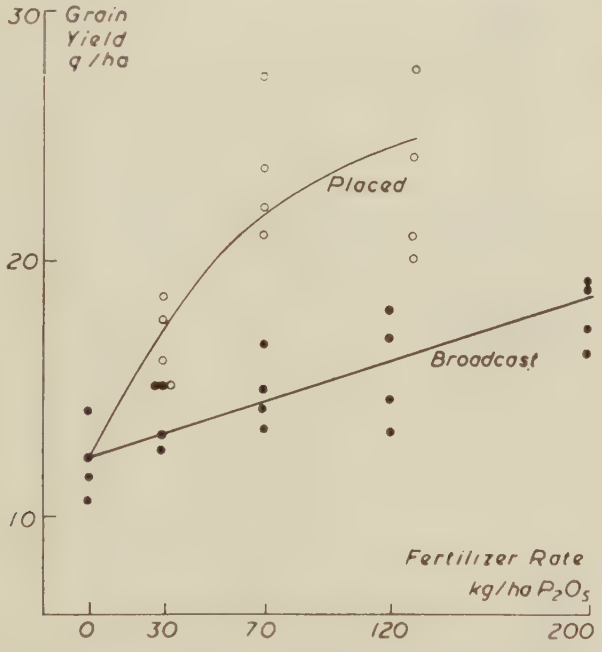

Fig. 3. Comparison between placement (○) and broadcasting (•) with phosphate for maize on sandy soil (Exp. Pr 1498).

Another example of the favourable effect of placement with superphosphate is given in Table I for oats. In this experiment placement was tested at different distances from the seed and at different depths. The result shows that deep placement (4 cm below the seed level) is more profitable than a shallow one and that a distance of about 4 cm in a horizontal direction gives the best results. Bands

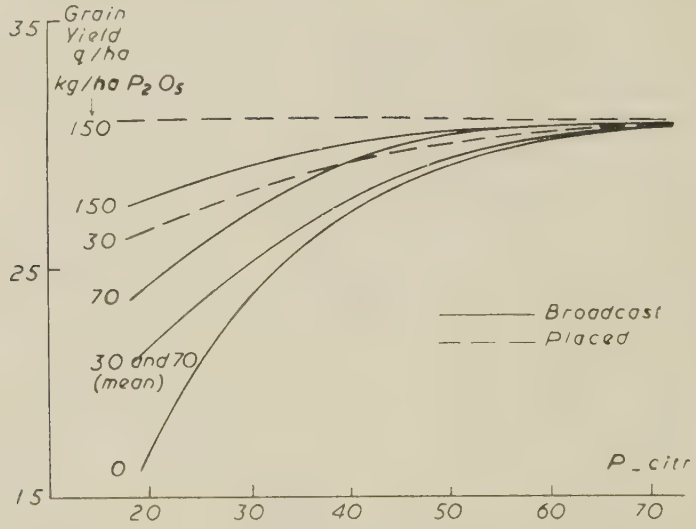

Fig. 4. Comparison between placement and broadcasting with phosphate for maize in dependence on P-citr on sandy soil (Exp. Pr 1482).

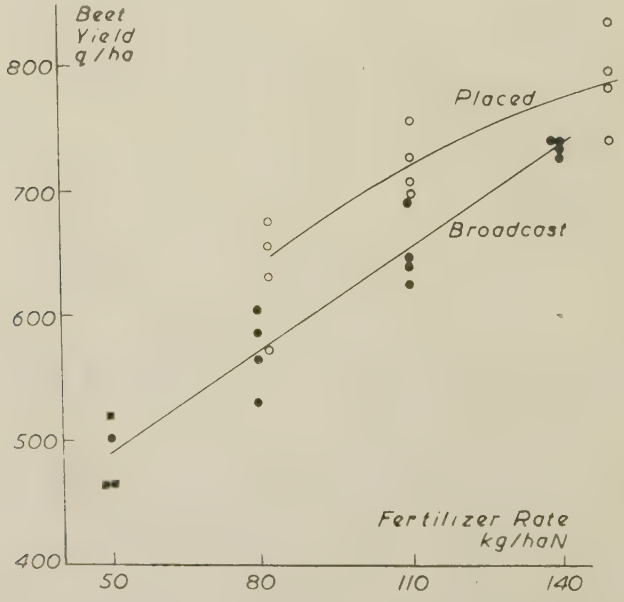

Fig. 5. Comparison between placement and broadcasting with nitrogen for beets on marine clay soil (Exp. Pr 1422).

placed at 8 cm from the seed are too far away and retard the early growth (Table II), the crop being unable to make up for this lag later on.

TABLE I

Yields of grain (oats) in q/ha on reclaimed heath soil (Exp. Pr 1052)						
Method of application	Distance*, cm	Depth **, cm	P_2O_5, kg/ha			
			0	40	80	200
Broadcast			0	8.6	15.9	23.7
Placed	4	0		10.0	16.3	14.9
	4	2		14.3	22.4	22.4
	4	4		19.1	20.9	23.4
Broadcast	8	0		10.5	14.4	19.2
	8	2		17.4	17.9	20.9
	8	4		18.9	16.2	21.9

* Distance of the fertilizer band from the seed.

** Depth of the fertilizer band below the seed.

TABLE II

Vigour score for oats on reclaimed heath soil on May 25 (Exp. Pr 1001)						
Method of application	Distance +, cm	P_2O_5, kg/ha				
		0	25	50	100	200
Broadcast		1½	2½	5−	6	8+
Placed	2		6−	8−	7½	8−
	8		3½	4½	6	6

\+ Distance of the fertilizer band from the seed.

TABLE III

Yields of grain (oats) in q/ha (Exp. Pr 1283)				
Method of application	N, kg/ha			
	20	40	60	80
Broadcast	18.5	24.2	32.0	37.8
Placed	18.6	28.8	36.1	38.3

Similar advantageous results are obtained by placing sulphate of potash on river clay deficient in potash for spring wheat and potatoes. Yields given by a single dressing of sulphate of potash placed in bands were about equal to the yields given by double dressings of broadcast fertilizer. Placing nitrogenous fertilizer also shows a

TABLE IV*a*

Relative value of placement in comparison with broadcasting (= 1.00) for **nitrogen** fertilizers

Cereals			Potatoes			Beets		
Soil		Relative value	Soil		Relative value	Soil		Relative value
Exptl. field	Type		Exptl. field	Type		Exptl. field	Type	
Pr 1283	sandy	1.35	Pr 1199	marine clay	1.00	Pr 1421	sandy	1.15
Pr 1284	sandy	1.00	Pr 1419	sandy	1.25	Pr 1422	marine clay	1.25
Pr 1285	sandy	1.55	Pr 1420	marine clay	1.25	Pr 1487	sandy	1.25
Pr 1286	sandy	1.65	Pr 1486	sandy	1.15	Pr 1490	marine clay	1.15
Pr 1290E	marine clay	1.15	Pr 1489	marine clay	1.15			
Pr 1336	marine clay	1.35	Pr 1602	sandy	1.15			
Pr 1337	marine clay	1.25	Pr 1612	marine clay	1.00			
Pr 1338	sandy	1.25						
Pr 1367	sandy	1.00						
Pr 1413	sandy	1.55						
Pr 1414	sandy	1.00						
Pr 1415	marine clay	1.15						
Pr 1416	marine clay	1.25						
Pr 1485	marine clay	1.15						
Mean		1.25			1.15			1.20

TABLE IV6

Relative value of placement in comparison with broadcasting (= 1.00) for **phosphorus** fertilizer

Cereals				Potatoes				Beets				Maize					Pulse crops				
Soil			Rel. value	Soil			Rel. value	Soil			Rel. value	Soil			Relative value **)		Soil			Relative value **)	
Exptl. field	Type	P-citr. *)		Exptl. field	Type	P-citr. *)		Exptl. field	Type	P-citr. *)		Exptl. field	Type	P-citr. *)			Exptl. field	Type	P-citr. *)		
Pr 1001	reclaimed heath	9	1.65	Pr 1198	marine clay	21	1.00	Pr 1148	sandy	28	1.00	L 1337	sandy	11	1.00	(4.00)	Pr 1146	sandy	28	5.00	
Pr 1002	sandy	28	3.35	PO 409	ferrous sandy	26	1.65	WB 1893	marine clay	18	0.50	OB 3141	ferrous sandy	23	--††	(5.00)	Pr 1147	sandy	28	10.00	
Pr 1052	reclaimed heath	4	2.50	OB 3139	ferrous sandy	42	2.00	ZGr 1004	ferrous sandy	26	1.00	Pr 1423	sandy	18	2.20	(2.50)	ZGr 972	sandy	23	--††	(7.00)
Pr 1053	marine clay	15	2.50	WB 1890	river clay	14	0.50	OO 1502	ferrous sandy	12	1.65	Pr 1424	sandy	32	4.00	(5.00)					
Pr 1056	marine clay	20	5.00	Pr 1366	sandy	28	e† 4.00	ZGr 1148	ferrous sandy	10	2.00	NL 98	sandy	29	1.00	(2.00)					
Pr 1057	marine clay	29	4.00	Pr 1366	sandy	28	l† 1.55	NOB 492	river clay	10	1.00	Horst	sandy		--††	(5.00)					
Pr 1058	sandy	15	1.80	Pr 1425	marine clay	18	1.00					Pr 1498	sandy	23	5.00	(10.00)					
Pr 1064	ferrous sandy	7	1.80	ZGr 1000	ferrous sandy	7	2.00					Pr 1499	sandy	23	--††	(5.00)					
Pr 1065	loess	7	2.20	NL 99	ferrous sandy	29	2.50					OO 1504	sandy	44	1.65	(3.35)					
Pr 1066	loess	14	2.20	ZGe 889	river clay	9	1.00					MB 147	sandy	45	--††	(4.00)					
Pr 1067	loess	15	1.80	VPr 250	sandy limed	24	4.00					MB 148	sandy	17	--††	(4.00)					
Pr 1138	reclaimed heath	6	1.00	ZGr 1149	ferrous sandy	10	2.00					NL 200	sandy	20	5.00	(2.50)					
OB 3250	ferrous sandy	15	2.00									NL 220	sandy	20	2.50	(2.50)					
ZGr 1003	ferrous sandy	10	3.35									NOB 485	sandy	22	4.00	(2.00)					
Pr 1491	reclaimed heath	8	1.55																		
Mean			2.45				1.90				1.20				2.90	(4.05)				7.50	

* P-citr = soil-P soluble in 1% citric acid.
** In parentheses: relative value calculated from vigour scores.

† e = lifted early, l = lifted late.
†† Without phosphate response or not harvested.

TABLE IV*c*

Relative value of placement in comparison with broadcasting (= 100), for **potash** fertilizer

Cereals			Potatoes										Beets		
River clay soils			River clay soils, *low* pH					River clay soils, *high* pH					River clay soils		
Exptl. field	K–HCl *)	Rel. value	Exptl. field	K–HCl *)	pH (KCl) **)	$CaCO_3$ content	Rel. value	Exptl. field	K–HCl *)	pH (KCl) **)	$CaCO_3$ content	Rel. value	Exptl. field	K–HCl *)	Rel. value
Pr 1068	0.010	4.00	Pr 1209	0.012	5		0.50	Pr 1210	0.013	7.1	–	2.20	Pr 1488†	9††	1.00
Pr 1200	0.010		WB 1901	0.010	5.1		1.00	WB 1898	0.010	7.15	–	2.00	Pr 1608	0.011	1.00
Pr 1201	0.012	3.35	Pr 1341	0.009	5.3		1.00	WB 1899	0.010	7.4	9.6	1.00	Pr 1609	0.012	1.00
Pr 1203	0.013		U 862	0.013	4.85		0.50	Pr 1342	0.012	6.9	2.7	1.45	Pr 1610	0.016	1.00
			NOB 465	0.012	4.05		1.00	WB 1982	0.014	7.2	3.7	1.00			
			NOB 466	0.009	4.25		1.00	WB 1984	0.009	7.1	6.5	1.65			
			Pr 1604	0.011	4.6		1.00	WB 1985	0.011	7.05	6.8	1.65			
			Pr 1606	0.014	4.7		1.00	WB 2030	0.012	7.3	7	2.20			
			VPr 250	0.011	5.25	0.1	2.00	WB 2031	0.010	7.6	13	1.55			
								WB 2032	0.019	7.6	11	2.20			
								Pr 1428	0.013	7.55	0.7	1.00			
								WB 2170	0.010	7.25	7	1.25			
								Pr 1607	0.014	7.0	–	1.00			
								VPr 250	0.012	6.75	1.2	2.00			
								WB 2273	0.012	7.25	5	1.55			
Mean		3.65					1.00					1.60			1.00

* K–HCl = soil potash soluble in 0.1 *N* HCl.
** pH(KCl) = pH measured in 1 *N* KCl.

† sandy soil.
†† K-number = soil potash soluble in 0.1 *N* HCl related to the soil humus content.

marked effect, though less than that of phosphate and potash fertilizers (Table III for oats and Fig. 5 for beets).

Placement of nitrogenous fertilizer prolongs the period of growth of potatoes and beets compared with broadcast manuring (see Plate I); in regions with a long and mild autumn the resulting increased assimilation period can significantly increase the yield.

If nitrogenous fertilizer at high rates is placed too near the seed (at a distance less than 2 cm for cereals and 5 cm for beets) germination is adversely affected on sandy soils because of the excessively high concentration. Heavy dressings of nitrogen (200 kg N per ha) in bands can also retard the early growth of potatoes; later, however, the crop grows more quickly than with broadcast fertilizer. In contrast, placing phosphate and potash fertilizers never caused any injury to the crops in our field trials. Preliminary pot experiments showed that the germination of the crop was severely injured when the fertilizer was drilled in contact with or directly below the seed, especially when highly soluble fertilizers (nitrochalk and sulphate of potash) were used on pulse crops and beets. For cereals, bands placed 2 cm and for other crops, 5–7 cm to the side of the seed, were safe in avoiding injury.

A summary of the results of all experiments is given in the Tables IV*a*, *b* and *c*, for nitrogen, phosphate, and potash respectively. In these tables comparative values are presented, being the ratio between the amounts of fertilizer required to give the same yield for broadcasting and placement. If, in the case of placement for example, a dressing of g kg of fertilizer per ha gives the same yield as a $5/2\,g$ kg per ha by broadcasting, then the value for placement in comparison with broadcasting is 2.5, which means that placement gives a saving of 60% on the fertilizer. In such a case the relation between yield y and rate of fertilizer x may be represented by $y = f(ax)$ for placement, when this relation is $y = f(x)$ for broadcasting. Therefore if in one graph, the yields for placement are plotted against the fertilizer dressings and the yields for broadcasting are plotted against the fertilizer dressings divided by a, the two arrays of points will determine the same curve $y = f(x)$. The correctness of the assumption that the curve $y = f(x)$ for broadcasting and $y = f(ax)$ for placement are the same, will be considered later on.

The transformation of the abscissa of the curve for broadcasting is carried out as follows. The yields for broadcasting are plotted against the fertilizer dressings on a glass plate. This array of points is projected vertically by parallel light(sunlight) onto an underlying graph representing the relation

between yield and rate of fertilizer by placement. By rotating the glass plate about the ordinate (fertilizer dressing zero) the projected points are adjusted to coincide with the array of points on the placement graph (projective transformation).

Tables IV*a*, *b* and *c* show that the value of placement in comparison with broadcasting depends in the first place upon the nature of the plant nutrient. For nitrogen application, this value (as a mean of all experiments) was found to be 1.20 for cereals, potatoes and beets. In the case of phosphate it was 7.50 for pulse crops, 2.90 for maize, 2.45 for cereals, 1.90 for potatoes, and 1.20 for beets, whilst in the case of potash on river clay it was 3.65 for cereals, 1.00 for potatoes at low pH and 1.60 at high pH, and 1.00 for beets *.

These figures show clearly that applying nitrogenous fertilizer in bands is more effective than broadcasting; phosphorus and potassium fertilizers sometimes give even more spectacular results when placed in the same way near the seed. This may be attributed to the rather small uptake of phosphate and potash when broadcast as a result of fixation; a localized concentration of fertilizer reduces fixation to a minimum and will therefore have a favourable influence on uptake.

The effect of placed superphosphate is greatest with pulse crops, followed by cereals and maize with a somewhat lower advantage. Placement of phosphate for potatoes and particularly for beets is less profitable. The effect of placed sulphate of potash is again less for potatoes than for cereals, whilst for beets placement has no advantage whatever. Cooke [3] relates these differences in the effect of placement for different crops to the nature of the root system. In his opinion placement is of particular value for rapidly growing crops with a limited root range (cereals and pulse crops); crops with an extensive root system (potatoes and beets) utilise nutrients more efficiently in the soil between the rows and show smaller benefits from a localized concentration of fertilizers. An investigation by van der Paauw [11] demonstrates the different behaviour of oats and potatoes with respect to an uneven distribution of fertilizer. Oats withdraw nutrients locally because of their relatively small root system; thus a distance of 8 cm from the plant to the fertilizer is an appreciable barrier. In contrast, potatoes with a well

* These figures differ slightly from those given in a previous paper [13], because somewhat more data are now available, and the treatment of the results of some experiments has improved.

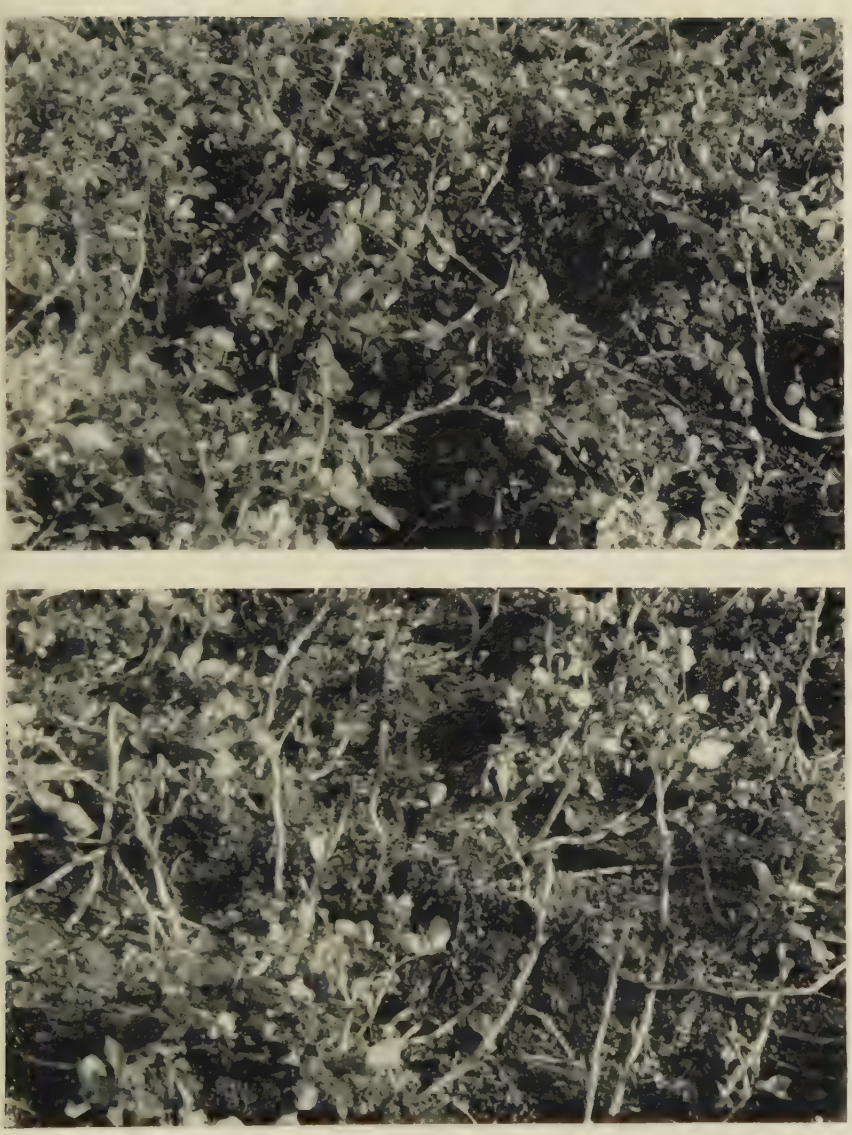

Plate I. Influence of placement of nitrogen on dying off of potatoes (Pr 1489, marine clay soil). **A**: 110 kg N per ha, placed. **B**: the same amount, broadcast.

developed lateral root system can take up nutrients situated further away and are therefore not hindered by an uneven distribution of fertilizer. Our investigations confirm the interpretations of Cooke with the exception of the results with maize. This crop has an extensive root system, yet placing superphosphate proved much better than broadcasting. It is well-known that in the early stages of growth maize can suffer severely from phosphate deficiency. Although some of the first formed roots grow in a more or less horizontal direction, they may not quite reach the broadcast fertilizer when shallowly incorporated; the nodal roots generally grow deeply and are unable to take full advantage of the fertilizer when broadcast.

Our experiments with potatoes showed a better effect of placement of superphosphate only on iron-rich soils. An experiment on a sandy soil (VPr 250, see Table IV*b*) also indicated that placement is of particular value when the availability of soil phosphate is low; in this experiment the phosphate nutrition was originally fair but was markedly decreased by liming, as indicated by the P-water value (van der Paauw [12]). This deficiency in phosphate resulted in a markedly decreased yield and a phosphate dressing increased the yield considerably, particularly in the case of placement.

There are indications that the advantage of placement of sulphate of potash for potatoes on river clay is greater at a high than at a low pH; it is known that the availability of potash for this crop is extremely low at high pH (Ferrari [4]). No advantage of placement over broadcasting was found with potash for beets, either on a non-fixing sandy soil or on a river clay soil deficient in potash.

To test the hypothesis H_0, that, if the relation between yield y and fertilizer dressing x for broadcasting is $y = f(x)$, this relation is $y = f(ax)$ for placement, with constant a for a given experiment, the method of m-rankings of Friedman was used (see Kendall [9]).

Per experiment the abscissa for broadcasting was transformed and a common curve was constructed through the resulting arrays of points for both methods of application. The deviations of the mean yield per dressing from this common curve were determined (with the proper sign). These deviations were ranked per experiment separately for broadcasting and for placement, and the ranks per dressing were added up over all experiments. From the rank sums

the coefficient of concordance W was calculated in the ordinary way and tested for significant difference from zero (Table V), column "common curve", row *a* and *b*). No distinction is made between crops because the numbers of field trials for the different crops were too small for individual testing, and there is no reason to suppose that the crops should behave differently in this respect. The experiments with potash have been added to those with phosphate, again owing to the small number of experiments. The experiments with 3 and with 4 fertilizer levels have been kept separate.

TABLE V

Levels of significance p of the rankings for the common curve and for the placement curve

Deviations	Nitrogen				Phosphate and potash			
	Common curve		Curve for placement		Common curve		Curve for placement	
	3*	4*	3	4	3	4	3	4
a. Broadcast-yield minus curve	0.60	0.31	0.54	0.38	0.53	0.32	0.58	0.32
b. Placement-yield minus curve	0.02	0.38	0.11	0.44	0.35	0.48	0.48	0.66
c. Broadcast-yield minus curve and curve minus placement-yield	0.10	0.60	0.11	0.66	0.26	0.79	0.36	0.97
Number of data	19	20	19	20	27	31	27	31

* Number of fertilizer levels.

If the hypothesis H_0 is not true, the mean yields for some or all levels of dressing of one or both methods of application will deviate systematically from the mean curve and so the rankings of the deviates will not be random permutations, *i.e.* the coefficient of concordance will show a significant difference from zero. From Table V the column for "common curve" shows, that only in the case of nitrogen the means for placement indicate that there are not quite random deviations from the common curve.

As the common curve was drawn through the array of points for both broadcasting and placement, systematic deviations may be expected not to be very large. Therefore the same calculations were made for deviations from the curve drawn only through the array of points for placement. Of course the means for placement gave not-significant values of W, indicating only that the curves were really drawn through the points; but the means for broadcasting gave also not-significant values of W (Table V, column "curve for placement", row *a* and *b*). This result renders our hypothesis more

evident, because in this test the position of the treatment means for broadcasting is examined by means of a curve which is entirely independent of the array of points for broadcasting.

To strengthen these two results and to discover especially if the curves for broadcasting and for placement both crossed the common curve, the rankings for broadcasting were calculated for the deviations "mean yield minus yield according to curve" and those for placement for the deviations "yield according to curve minus mean yield"; these $2n$ rankings (for n experiments) per level of dressing were afterwards added and from these sums the coefficients of concordance was calculated. Here again no significant values of W were found (Table V, row *c*).

As the coefficient of concordance W was just significant different from zero only in one out of 24 (although not quite independent) cases, the assumption that the effect of g kg of a fertilizer per ha if placed is aequivalent to ag kg per ha if broadcast, whatever the value of g, would therefore appear valid. The general form of the yield curves can thus be described as follows: broadcasting and placement give the same maximum yield, which, in the case of placement however, is attained at a lower level of fertilizer.

4. THEORETICAL CONCEPTS OF PLACEMENT BY DE WIT

The conclusion at the end of the preceding section required a critical consideration of the theory of de Wit [18]. According to this theory the determination of a constant ratio between the effect of the two methods of application for the whole fertilizer dressing—yield curve, as made by us, would not be possible.

De Wit has tried to gain an insight into the underlying principles, in order to predict the effect of placement under definite conditions; he considers the uptake of nutrients as the main factor, since in general, the yield is determined by this. The theory may be summed up as follows.

If x_b is the distance between the crop rows and M_b the amount of broadcast fertilizer in kg per ha, the uptake in this case is compared with that for placement at a level of $\frac{x_r}{x_b} \times M_b$ kg/ha, were x_r is the width of the fertilizer band

In this comparison the concentration of fertilizer per unit area of

fertilized soil is the same for the two methods of application, hence the reaction between soil and fertilizer is also the same. Therefore the uptake for the two methods of application can only be compared from the time when the roots have developed over the whole area; the ratio between the amounts of roots in the fertilized soil should then agree theoretically with the ratio in volume of fertilized soil exploited.

There is, however, an important difference in uptake, owing to the fact that with placement a smaller part of the root system is in contact with fertilized soil. Goedewaagen [6] pointed out that it is not necessary for the fertilizer to be in contact with all the roots; it is sufficient that the requisite quantity of plant nutrients is available to part of the root system. A compensation of uptake will then occur, resulting in an increasing uptake in the bands per unit area of roots (physiological compensation). Furthermore this process is often augmented by a more vigorous root development in the bands (morphological compensation). De Wit has applied this phenomenon of compensation in the elaboration of his theory.

In Fig. 6 * the curve C represents the relation between the ratio of the fertilized area (x_r/x_b) and the ratio of uptake (U_r/U_b) for the two methods of application, when the concentration in the fertilized soil is the same. De Wit has computed this relation from experiments (pot and field trials) encountered in the literature.

Designating the uptake, when M_b kg fertilizer per ha is broadcast, by U_b kg per ha, it is found that as a result of compensation the uptake is 0.75 U_b if 0.5 M_b is placed in bands with a width equal to half the row distance ($x_r/x_b=0.5$). Starting from the rate—uptake curve for broadcasting, the curve for placement can be constructed by multiplying the uptake for broadcasting by 0.75 (Fig. 7). This assumes that the uptake ratio between the two methods of application is independent of the concentration of the fertilizer.

It is characteristic of Fig. 7 that the uptake curve for placement intersects that for broadcasting, so that the uptake in the case of placement at low fertilizer levels is more, but at high fertilizer levels is less than that for broadcasting. The point of intersection shifts to lower rates as the band width is decreased.

From the uptake curves (known for broadcasting from experience

* The Figures 6—8 have been taken from the publication of De Wit [18].

and calculated for placement by means of the compensation function in dependence on the band width) and the relation between uptake and yield, the rate—yield curve for placement can be calculated. The uptake—yield relation is usually independent of the method of application, exept in the case of antagonistic effects: if

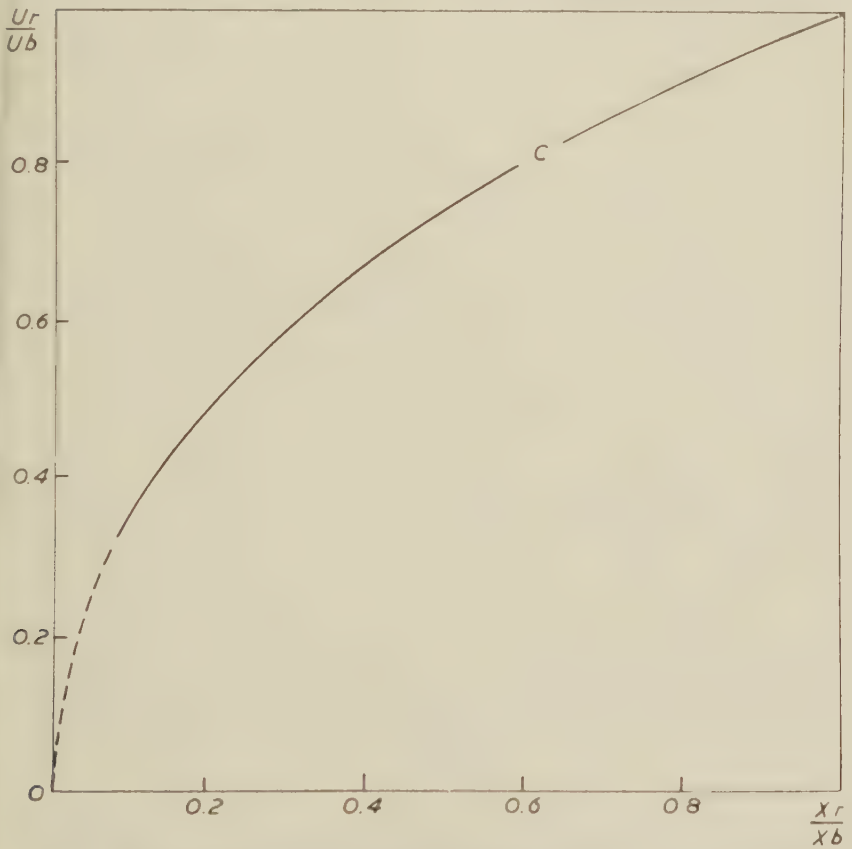

Fig. 6. Relation between the ratio of fertilized area (x_r/x_b) and the ratio of uptake (U_r/U_b) for placement and broadcasting according to de Wit [18].

application of the fertilizer decreases the availability of some other nutrient, the effect, according to de Wit, is less serious when the fertilizer is placed than when it is broadcast. Thus the yield corresponding to a certain quantity of the fertilizer nutrient absorbed by the plant, will be greater with placement than with broadcasting.

The reverse situation is also possible. Fig. 8 represents the four possibilities, according to de Wit.

An important conclusion from this theory is that higher returns can be obtained by placing light dressings in narrow bands, but that heavy dressings in narrow bands produce lower yields than broadcasting; therefore the width of the fertilizer band should not be too narrow.

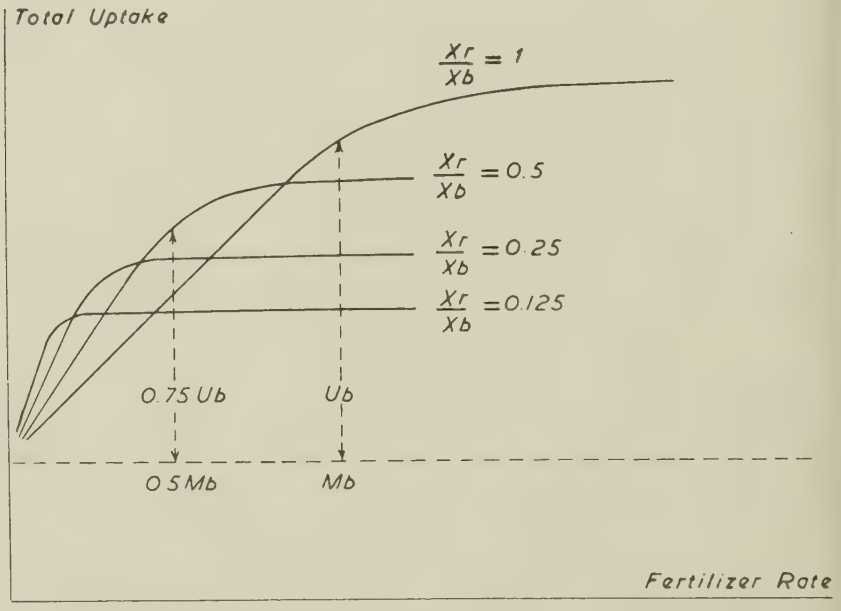

Fig. 7. Relation between fertilizer rate and uptake for placement and broadcasting in dependence on the width of the fertilizer band according to de Wit [17].

5. TESTING THE THEORY OF DE WIT AGAINST THE RESULTS OF OUR EXPERIMENTS

We now return to our own investigations. It is clear that according to de Wit a transformation of the abscissa of the curve for broadcasting as done by us will not be admissible because, in 3 out of the 4 possible cases as shown in Fig. 8, the two curves are of different shape. After transformation the treatment means would be situated systematically about the common curve. In Section 3 however it has

been shown that the curves may be considered as similar. Only Fig. 8, 1a (2nd quadrant) provides a correct representation.

In giving a definite judgement on the theory of de Wit, the presence of the point of intersection between the rate—uptake curves for placement and for broadcasting (Fig. 7) is decisive. This intersection is characteristic of all the examples given in Fig. 8 and would occur, if the supposition that the compensation is independent of the concentration should be correct.

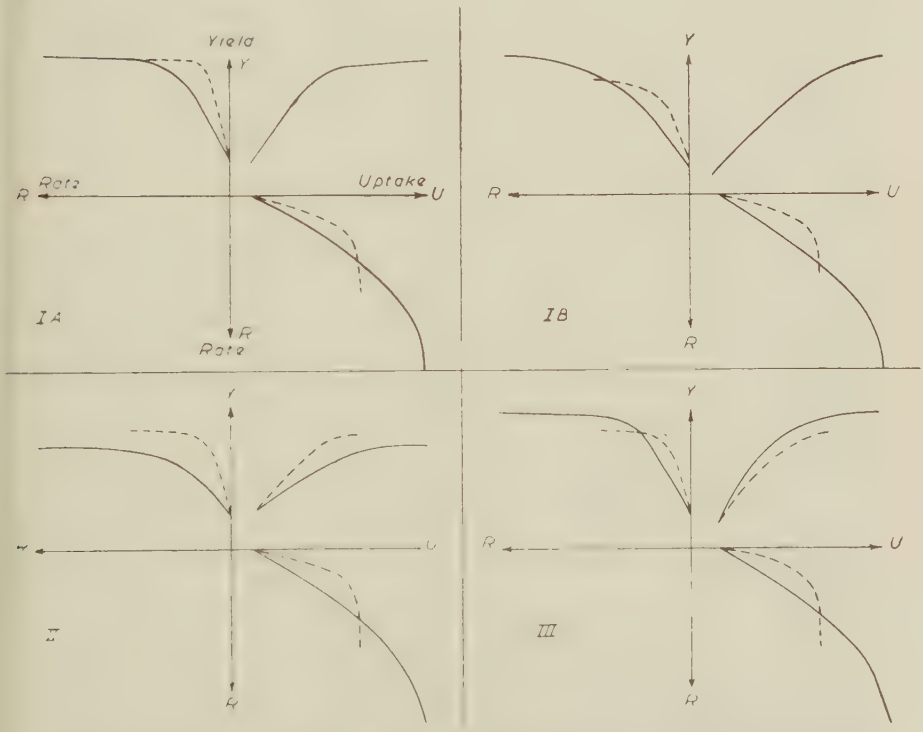

Fig. 8. Relation between fertilizer rate (R), uptake (U) and yield (Y) for placement and broadcasting according to de Wit [18]. Cases IA and IB without, cases II and III with antagonistic reaction (——— broadcasting, - - - - - placement).

This intersection, however, has seldom been found in our experiments, although the fertilizer band in our experiments is narrower than that with which de Wit has worked in developing his theory ($^1/_5$—$^1/_8$ the distance between the crop rows in our experiments and

1/4 in the case of de Wit), so that it would certainly have been expected, if the suppositions of de Wit are valid.

So as to be quite definite about this point, the observations themselves have been used in the sign test. This test is carried out for the experiments with both phosphate and potash. The experiments with nitrogen are omitted because there were rather a large number of cases in which the two rate—uptake curves were linear; in this case the result would turn out too favourably for our claim that there is no point of intersection. For each fertilizer dressing the number of cases in which the uptake for placement exceeds that for broadcasting is determined. With increasing fertilizer dressings this, as a percentage of the total number of cases, turns out to be 82, 75, 71 and 56 (number of cases 27, 28, 24 and 25, respectively). The lower confidence limits (one-sided symmetrical, $p = 0.95$) are 64, 57, 51 and 38%, respectively.

Generally speaking the uptake for placement exceeds that for broadcasting. According to the above lower confidence limits an intersection, if any, would occur above the third * level of fertilizer dressing (except for a probability of 5% **). De Wit's conclusion concerning the minimum width of the fertilizer band (about one-fourth of the distance between the crop rows), based on the presence of this intersection at a normal fertilizer dressing, has therefore to be rejected in practice.

It is possible that compensation increases with the concentration, so that the maximum uptakes obtainable from placement and broadcasting are the same. The experimental foundation of the theory of de Wit should be strengthened by an inquiry into the dependence between compensation and concentration.

It is also possible that a comparison between uptakes for the two methods of application in field trials as made by de Wit is inadmissible. Broadcast fertilizer will be localized shallowly in rather dry soil. In this case the uptake will fall behind that with placement where the fertilizer lies deeper. In this connection a study of the

* In our experiments this fertilizer rate varied from 80–140 kg P_2O_5 or K_2O per ha (in the case of potash for root crops, to 200 kg K_2O per ha), respectively.

** Our conclusion as to the situation of the point of intersection could be drawn with satisfactory reliability by using the simple, though not very powerful sign test, which only distinguishes between high and low uptakes. A check by a more powerful test is unnecessary for this reason.

influence of depth of application and the moisture status of the soil on uptake is desirable.

Another objection is that the significance of the uptake during early growth, before the roots have developed under the entire soil surface, may have been underestimated by de Wit. He assumes that the yield is closely related to the total quantity of plant nutrients taken up; this implies that the nutrient contents are the same when a kg fertilizer per ha applied by placement gives a yield as high as b kg fertilizer per ha applied by broadcasting ($a < b$). However, chemical analysis of the crop in this case showed lower nutrient contents with lower level of fertilizer applied in bands. Therefore an equal yield is obtained with a smaller quantity of the fertilizer nutrient absorbed by the plant; this would suggest that the intensity of uptake as related to time is important. It is known that the growth of a crop benefits particularly from manuring in the early stages when the later observed differences in yield already mainly have been predetermined [2 7]. It is therefore presumed that in the case of placement, the crop, which, from the very beginning, is able to utilize the total quantity of fertilizer applied, obtains a start which initially determines the differences in yield. In the case of broadcasting however, some of the fertilizer is initially out of reach of the roots; later, when the roots permeate the whole space between the crop rows, more plant nutrients can then be taken up. In the case of equal yield this can give rise to a higher nutrient content in the plant compared with placement. Therefore a similar uptake of plant nutrients is not a condition for equal yields. Experiments with radioactive isotopes clearly show this fundamental difference in uptake between the two methods of application [5 8 10 15 17].

When such a difference does occur, de Wit assumes that in the case of placement, this is the result of a decreased antagonistic effect on some other plant nutrient resulting in a higher yield. We, however, are not satisfied by this conception as an explanation for the significant differences in yield, *e.g.* in the case of phosphate.

The ratio between the amounts of roots exploiting the fertilized soil at the critical moment when the roots have not developed completely under the entire soil surface, is not equal to the ratio between the width of the fertilizer band and the distance between the crop rows. A comparison between total uptakes at the same concentration from the time when the roots penetrate the whole

distance between the crop rows, is doubtful, because the influence of the time of uptake is left out of consideration. The importance of the ratio between the fertilized area of the soil and the compensation factor therefore becomes smaller.

SUMMARY

The results are given of an investigation in the Netherlands, made over a number of years, on placement of fertilizers. This new method of application has been tested in field trials, in which placement has been compared with the usual method of broadcasting at certain increasing levels of fertilizer. The experiments relate to nitrogen, phosphate and potash on different kinds of crops and soils.

Generally speaking, it would appear that placement is better than broadcasting. The difference between the two methods of application can be represented by a factor indicating the ratio between the amounts of fertilizer giving the same yield for both. Nitrogen applied in bands is about 1.20 times as effective as broadcasting for cereals, potatoes and beets; phosphate for pulse crops 7.50, for maize 2.90, for cereals 2.45, for potatoes 1.90 and for beets 1.20; potash on river clay for cereals 3.65, for potatoes 1.00 at low pH and 1.60 at high pH and for beets 1.00. There are indications that the prevention of fixation and a better early growth as a result of the localisation of the fertilizer near the seed at an adequate depth are reasons why placement is successful.

The conclusions of de Wit with regard to the effect of placement are not corroborated. It appears that placement gives higher yields at light dressings, and at heavy dressings yields as high as broadcasting, whereas, according to de Wit, a favourable effect would be attained, as a rule, with light dressings and an unfavourable effect with heavy dressings.

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CHROMATOGRAPHY AS A MEANS OF SELECTING EFFECTIVE STRAINS OF *RHIZOBIA*

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In selecting effective strains of *Rhizobia* the method usually followed is to inoculate sterile seeds of leguminous plants. After plants from these seeds have been grown for a sufficient period in a sterile culture medium, they are cropped and analysed for their nitrogen content. This method, though efficient, has some difficulties.

The medium in which the plantlets are grown may be an agar, a liquid or a sand medium. The agar medium may be successfully used for smaller seeds, such as clovers. Larger seeds, giving as a rule larger plants, are difficult to handle in agar tubes. In liquid media their nodulation will often be poor. In sand cultures, cropping the whole plants free from sand, especially the roots, requires a good deal of labour. For these reasons we have been looking for another method.

Considering that the nitrogen compounds, formed in the nodules, will be transported through the vessels to the growing parts of the plants, it should be possible to demonstrate the presence of these substances in the upward sap stream.

Since it would be expected, that amino-acids or possibly amides will occur among the first products of nitrogen fixation, it should be possible to detect these substances in the bleeding sap when the upper parts of the plants, inoculated with effective strains, have been cut off. Using chromatographical methods for analyses of this type, we started in the autumn of 1954 a series of experiments with good hopes of differentiating successfully between effective and ineffective strains of *Rhizobia*. The substances mentioned above would not be expected to be present in uninoculated plants, nor in

those inoculated with ineffective strains. Furthermore there might be differences in the amounts and in the number of nitrogenous substances, depending on the degree of effectiveness of the bacterial strain used in the experiment.

Theoretically this method might give some insight into the nature of the substances formed first in the process of nitrogen fixation, though many transformations may have taken place before the sample is taken from the bleeding sap.

Our experiments have been mainly carried out with peas, in a few of them we used lupins. The following, however, will deal exclusively with experiments with peas of the variety "Rondo".

The plants have been grown from seeds the surface of which was sterilised by shaking them for 10 minutes in concentrated H_2SO_4, which was washed off with sterile water, followed by a 10-minutes treatment with a sodium hypochlorite solution and a final washing with sterile water. The seeds were then pregerminated on agar plates and only those that gave sterile sprouting seeds were used in the experiments. These seeds were planted in an artificial soil composed of washed river sand with 4 per cent of peat litter and 0.03 per cent of Crone's salt mixture without nitrogen. To this salt mixture some minor elements (Zn, Mn, B, Mo, Cu) were added. This soil was put into glass jars of 1.5 litre capacity (Fig. 1), in which a perforated metal plate at the bottom had been covered with glasswool. A

Fig. 1. Jars for sterile culture of plants

bent glass tube, filled with fine gravel and closed with a cotton plug was inserted into the soil.

These jars, after having been covered with a sheet of cellophane paper, were steam sterilised at 120°C for a period of 2 hours. After having cooled down to room temperature the pregerminated seeds were planted and immediately covered with about 1 cm of sterile paraffined glass sand *. As a rule 3 plants were grown in each jar, kept in the greenhouse in daylight and, if necessary, supplied with artificial light (Philips high pressure mercury lamps). The weight of the jars was kept constant throughout the experiments by adding sterile tap water through the bent glass tube. The amount of water added during this period was measured.

When the plants had grown sufficiently, they were cut off 1.5 to 2 cm above the soil. A piece of rubber tubing, into which a length of capillary glass tube was inserted, was placed over the stump. After a period of 4 to 7 hours the glass tube became partly filled with bleeding sap (Fig. 2). Light and moisture during the growth

Fig. 2. Collecting of bleeding sap

of the plant and effectiveness of the strain used seem to be important factors affecting the rate and the amount of sap delivered by the cut-off plants. At first we had some difficulties in obtaining enough

* This method is a simplification of the method used by Gerretsen (Das Katadyn-Verfahren zur sterilen Kultur höheren Pflanzen. Planta, Arch. Wiss. Botanik **23**, 593–603 (1935)).

sap to make our chromatograms, especially in winter, but in summer the plants bled more easily. Uninoculated plants and plants inoculated with ineffective strains did not bleed as freely as those inoculated with effective strains.

Usually the sap of 6 plants was collected and this mixed sap was used for our chromatograms on Whatman no. 1 sheets, measuring 46 by 57 cm. Several tests were run on one sheet of paper, usually together with a mixture of known amino-acids As a solvent we generally used phenol-water in a one-dimensional descending chromatogram. To prevent oxidation of the phenol a knife-point of KCN was added to a dish of water at the bottom of the chromatography box[3 4]. In the same dish we put 2% of NH_3 [3] to separate the spots of the basic amino-acids more clearly. Another dish at the bottom was filled with phenol-water.

Before use the phenol was purified by the method of Draper and Pollard [5].

After drying the chromatogram at a temperature of 70°C it was developed by spraying with a 0.15% solution of ninhydrin in chloroform [10]. Some collidine was added to the spraying solution [7 10].

Schematically the type of chromatogram obtained is shown in Plate I.

The chromatograms of about 38-day plants inoculated with effective strains, always showed a blue spot of aspartic acid, a yellow-brown spot of asparagine and a purple spot of glutamine. We never found a spot of glutamic acid. Blanks show no spots at all or a very faint one of aspartic acid. Ineffective strains gave a spot, mostly rather weak, of aspartic acid. This, however, holds only for a bleeding period of no more than 4 to 7 hours and with amounts of sap not exceeding 0.1 ml. If the sap is gathered after a bleeding period of 24 hours with effective strains more spots appear; for instance we often found spots in the positions of valine and leucine. In some cases these spots were also found after a period of 4 to 7 hours. Ineffective strains after a bleeding period of 24 hours sometimes also showed spots in the positions of glutamine, valine and leucine. With blanks, however, these spots were never found. *Hence the presence of a spot of asparagine, readily recognisible by its colour, is a reliable indicator for effective strains.* This holds true for a bleeding period of 4–7 hours as well as of 24 hours. For glutamine it only is valid for a bleeding period of 4–7 hours.

Plate II shows a typical chromatogram as found with sap from 38-day old plants and a bleeding period of 4 to 7 hours. From left to right the sap from a blank, an ineffective strain, an effective strain

and a mixture of aspartic acid, asparagine and glutamine were run on this paper strip.

In all our experiments carried out with strains of Dutch, Swedish, Finnish and Belgian origin, we compared the results of the chromatographic method with the chemical analysis for total nitrogen and percentage of nitrogen in the dry matter of the above-ground parts of the plants. The roots were not analysed. Table I shows that when asparagine was present on the chromatogram rather larger amounts of nitrogen were always present as compared with experiments in which asparagine spots were absent.

TABLE I

Plants: 36 days old. Bleeding time: $6^1/_4$ hours				
No. of strain	Total N in the green parts of 6 plants in mg	N-percentage in the dry matter of the green parts	Asparagin	Water taken up in ml
Not inoculated	31.14	1.99	—	600
P_8	35.97	1.89	—	640
P_{16}	70.80	3.36	—	675
H_{VIII}	53.36	3.10	—	800
P_{RE}	114.02	4.28	+	1000
S_{310a}	117.53	3.68	+	1035
S_{313}	131.98	3.90	+	1005
P_{36h}	154.08	5.15	+	1000
P_{F2}	225.70	5.45	+	1040

To demonstrate the difference in transpiration between blanks, ineffective strains and effective strains, the amount of water added during the growth is recorded in this table. As might be expected the plants inoculated with effective strains evaporated much more water than the others.

The plants were grown for a period of 36 to 40 days, and, at this age, the plants showed rather clear differences in size and in colour. Hence it could be claimed that no analysis of any kind need be made to show the effect of the inoculation (Plate III). Therefore our next experiment made was to find the earliest moment at which differences in the chromatograms appear. Starting our experiments with seeds of 200 to 300 mg we did not find clear differences between the chromatograms of inoculated and uninoculated plants after a growth of 2 to 3 weeks. With plants of 4 weeks old, however, the differences were already remarkable. After a bleeding period of

Plate I (top left): Chromatogram schematically.

Plate II (top right): Explanation in text, p. 257

Plate III (bottom): Pea plants, from left to right: blanc, P_{16} (uneffective strain), H_{VIII} (uneffective strain), P_{F2} (effective strain), P_{RE} (effective strain).

4–7 hours giving 0.1 ml sap the effective strains showed rather good spots of aspartic acid, asparagine and glutamine (Plate II). In this stage of growth a difference in colour of the plants may already be observed, but differences in size of the plants and in nitrogen content were still not significant in our experiment.

To acheive a further shortening of the time during which the plantlets grew on the nitrogen present in the seeds, we now started with seeds weighing not more than 190 mg. With plants from these seeds we got differences in the chromatograms as early as 3 weeks after planting, at a time when there was no difference observable in the colour and size of the plants and only a very small and unreliable one in the N-content of the green parts. At this stage of development with effective strains the chromatograms showed spots of aspartic acid, asparagine and glutamine, whereas blanks and ineffective strains produced spots of aspartic acid only. *Hence with the method described here it is already possible to select effective strains after 3 weeks from planting.*

In another experiment we removed the cotyledons after germination of the seeds. No results were obtained however, as the plants grown from these sprouts did not produce enough bleeding sap.

As stated above all our experiments so far were made with 0.1 ml of bleeding sap, this being the minimum quantity which gives reasonably distinct spots. Our chromatograms were carried out unidimensionally, using phenol-water as the developing solvent. What would appear, however, if we used other quantities of sap, two-dimensional chromatography and other solvents? First we tried application of more sap in the chromatogram. With more sap at least one more spot was found, which appeared in the position of hydroxyproline and gave a yellow spot just below that of glutamine. An amount of 0.16 ml sap was sufficient to give this spot. In addition to spots of aspartic acid, asparagine, glutamine and hydroxyproline 2 faint spots were very often present in the positions of valine and leucine, but we are not yet sure of the real nature these spots.

On application of 2-dimensional chromatography using phenol–collidine we observed that the spot in the position of glutamine was separated into two by collidine. The R_f's of one of these spots

were identical with those of glutamine. The R_f-phenol of the other one lay between those of alanine and threonine, whereas the R_f-collidine was identical with the R_f's of these two amino-acids.

To identify this spot further, propanol and butanol—acetic-acid were used as solvents; the results of these solvents as well as the colour of the spot after spraying with ninhydrin points to threonine.

To corroborate this supposition we tried electrophoretic methods to separate the amino-acids of the sap. Applying 600 V and a Mac Ilvaine buffer (Na_2HPO_4 and citric acid) of pH 2.2, 400 ml diluted to 1 litre, we effected a separation in the sap of aspartic acid, glutamine, asparagine and 'threonine'. Control strips with these amino-acids separately as well as in a mixture, made it almost certain that these 4 amino-acids were actually present in the sap.The experiments on the identification of the spot of 'threonine' are continued.

When rather large amounts of the sap of blanks were treated electrophoretically in the same way 'threonine' was found to be present also *. This spot was also found in a chromatogram of 0.3 ml sap of blanks when run with propanol—water, butanol—acetic-acid—water or phenol—collidine 2-dimensionally.

Considering our observations so far we conclude that only a small number of amino-acids is transported through the vessels as detected by our technique. Others may be present in the sap, but only in such small quantities, that they escaped our observation. Among those found, aspartic acid, asparagine and glutamine are the most important. It is rather puzzling, that glutamic acid never has been found. Referring to papers by Burris and Wilson [2], Wilson [8], Wilson and Burris [9], Zelitsch *et al.* [11 12 13], this amino-acid should be among the first and most important formed in nitrogen fixation. Furthermore Hunt [6] found it to be present in cell sap of nodules together with 15 other known amino-acids and 3 unknown ninhydrin-sensitive substances. We assume, that the glutamic acid, formed in the nodules, is rapidly transformed into glutamine before being transported through the plants.

Still other amino-acids may be present, *e.g.* such as are found with difficulty using phenol and collidine. This is rather improbable

* For this section of our work we are greatly indebted to Miss M. H. Deinema and Mr. M. F. Kerkhof Mogot.

however, as we did not find any more spots with propanol-water (7 : 3 v/v) and butanol–acetic-acid–water (25 : 6 : 25 v/v)[10] and electroforesis. Neither did we find S-containing amino-acids by running with phenol after treatment with H_2O_2 and ammonium molybdate[3]. Nor were more amino-acids found after hydrolysis of the bleeding sap.

In our experiments with effective strains since we always found aspartic acid and asparagine to be present in fairly large quantities, the theory that aspartic acid is the first product of nitrogen fixation in leguminous plants is tempting, but we prefer to leave this point out of discussion.

SUMMARY

1. In 0.1 ml of bleeding sap of uninoculated plants aspartic acid was found to occur as the sole amino-acid usually in a small concentration. With larger quantities of sap we also found a spot in the chromatograms and in the electropherogram, that most probably is identical with threonine.

2. Under the same conditions with a bleeding period of 4—7 hrs. aspartic acid was the only amino-acid detectable in the chromatogram of the bleeding sap of plants inoculated with ineffective strains.

3. Sap of plants inoculated with effective strains contains aspartic acid asparagine, glutamine, hydroxyproline and 'threonine'. Sometimes in addition to these substances 1 or 2 unknown substances were found, having R_f-phenol values about identical with those of valine and leucine.

4. Glutamic acid never was detected in the bleeding sap. We suggest, that glutamic acid formed in the nodules might be rapidly transformed into glutamine and transported in this form.

5. The uptake of water is significantly higher with effective strains than with ineffective ones or with plants that have not been inoculated.

6. With this technique it is possible to select effective strains within 3 weeks after planting.

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BEHAVIOUR OF IRON CHELATES IN CALCAREOUS SOILS

I. LABORATORY EXPERIMENTS WITH Fe-EDTA AND Fe-HEEDTA

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INTRODUCTION

Soil applications of the ferric iron chelates of ethylenediaminetetracetic acid, (Fe-EDTA), and N-hydroxyethyl ethylenediaminetriacetic acid, (Fe-HEEDTA), have been used to control lime-induced chlorosis in plants (Bould [1 2], Leonard and Stewart [7] and Wallace *et al.* [12]).

Lunt and Wallace [8], Stewart and Leonard [11] and Wallace *et al.* [13] conclude from laboratory studies that the behaviour of such iron chelates in soils is complex, being influenced by the amounts of clay and calcium carbonate present. Radioactive iron has been used to follow the absorption of chelates by plants from the soil by Holmes and Brown [6] and Wallace *et al.* [13]. Although the latter have noted that isotopic exchange may occur with an aromatic amine chelate, Fe-138, of undeclared composition, no quantitative experiments have been reported that estimate the extent of the exchange between labelled chelates and soil iron, or the error involved in relying on isotopic analysis alone.

The laboratory experiments described below were designed to give more information on the behaviour of these two iron chelates in calcareous soils when applied at rates comparable with those used in the field. Solutions of Fe-EDTA and Fe-HEEDTA, labelled with Fe^{59}, were applied to samples of dry soil, which were then stored for various periods of time before being extracted with water. Iron in the extracts was estimated in three ways, namely the isotope method, direct measurement of iron chelate and by colorimetric

analysis. The chelates of other metals also present in the extracts were converted to iron chelates for estimation.

EXPERIMENTAL

1. *Soil*

The soil used was a mixture of equal parts of top-soil and sub-soil from an Evesham orchard in which fruit trees were showing symptoms of severe lime-induced iron chlorosis (Bould [1]). Before use the soil was air-dried and ground to pass a 2-mm sieve. The sieved soil contained 28% calcium carbonate and 35% clay; its pH was 7.8.

2. *Preparation of labelled chelate solutions*

The Fe^{59} was supplied by the A.E.R.E., Harwell, as a solution of ferric chloride in dilute hydrochloric acid, at a specific activity of 3 μc Fe^{59}/mg Fe. This was combined with further inactive ferric chloride to give the two carrier levels. Equimolar quantities of the chelating agents were then added as their sodium salts. The pH of the iron chelate solutions was adjusted with sodium hydroxide to a value of 6 before application to the soil.

3. *Treatments*

Experiment 1. Seventy-five 50-g samples of soil were prepared and to each was given one of the following five treatments as 20 ml of solution:

(1*a*) 0.5 mg iron as Fe-EDTA
(1*b*) 5.0 mg ,, as ,,
(1*c*) 0.5 mg iron as Fe-HEEDTA
(1*d*) 5.0 mg ,, as ,,
(1*e*) Water.

0.2 μc Fe^{59} was included in the 20 ml of solution applied as treatments 1(*a*) — 1(*d*).

After each of the five time intervals, (1, 2, 4, 8 and 15 days respectively), three replicates of each treatment were extracted with water. While awaiting examination the treated soils were stored in glass jars with loose cardboard covers, except those stored for 15 days which were kept in bottles with corks sealed to maintain constant moisture.

Experiment 2. Some anomalous results were obtained in the first experiment from the soil stored in sealed containers. A comparison was made, therefore, of the behaviour of the chelate—soil system

when allowed either a free or a limited air supply during storage. Thirty-six 50-g samples of soil were prepared and to each one of the following six treatments was given:

5.0 mg iron as Fe-EDTA,	sealed	(2*a*)
	unsealed	(2*b*)
5.0 mg iron as Fe-HEEDTA,	sealed	(2*c*)
	unsealed	(2*d*)
Water	sealed	(2*e*)
	unsealed	(2*f*)

No radioactive iron was used in this experiment.

One of each pair of treated soils was left in a jar with loose covers as before, the other in a small bottle with the cork waxed to give an air-tight seal. Three replicates of each of the six treatments were extracted with water after eight days, the remainder after fifteen days.

In both experiments all treatments were applied as 20 ml of solution, this being sufficient to bring the 50 g dry soil to approximately its moisture equivalent. All the containers were stored in the dark, the temperature range being 15–22°C.

4. *Extraction procedure*

At the appropriate time the soil was transferred to centrifuge bottles (250 ml) with 100 ml of water. The bottles were shaken mechanically for 30 minutes and then centrifuged, (2500 rpm for 15 minutes). The supernatant liquid, approximately 100 ml, was clear and suitable for analysis without further treatment.

5. *Methods of analysis*

a. Soluble iron

(i) Isotope method. All radioactive assays were carried out on 10 ml aliquots by means of a scintillation counter. Standard solutions, prepared from those used for the treatments, were counted after every three or four determinations. The soluble iron, isotope method, (Table I), is the amount of iron that was originally associated with the radioactivity, found in the extract calculated from the observed count on the sample and its initial specific activity.

(ii) Colorimetric method. The iron was determined colorimetrically using *o*-phenanthroline (Fortune and Mellon) [4]. This method has been shown

to be valid in the presence of EDTA and HEEDTA by Wallace *et al.* [13]. The iron extracted from the soil in the water treatments was used as a "blank".

(iii) Spectrophotometric method. The chelated iron was estimated by the ultra-violet spectrophotometric method (Hill-Cottingham) [5]. The mean optical density of the extracts from the water treatments was used as a "blank".

It was assumed that, of the other metal chelates present in these soil extracts, calcium would predominate and experiments here showed that there was no absorption due to the calcium chleates at the wavelengths used in this method for iron.

b. Total chelate

The extract was acidified and an excess of ferric ions added to convert the calcium chelate into iron chelate. The subsequent increase in chelated iron concentration, measured as in para. *a*(iii) above and corrected for the absorption due to excess ferric ions, was then proportional to the original concentration of calcium chelate. The figures quoted as total chelate in Tables I and II are the sum of the iron equivalent of the chelated calcium and of the iron chelate values in the adjoining columns.

6. *Presentation of results*

The results presented are those obtained from analysis of the 100 ml of extract, but multiplied by a factor of 1.2, on the assumption that there was a further 20 ml of similar solution left in the soil after decantation of the clear centrifugate.

RESULTS

The results of Experiment 1, given in Table I show that the amount of soluble iron falls at successive times of extraction. However at both levels of Fe-EDTA treatment, the 15-day results, (from containers sealed to retain moisture) are anomalous, being greater than the corresponding figures obtained after 8 days.

Table II gives the result of Experiment 2 and shows that more soluble iron was present in the extracts from the sealed containers than from the unsealed, this effect being most marked with the Fe-EDTA treatments.

Fig. 1 shows the amount and composition of the chelate recovered from the soils in unsealed containers, while the change in the ratio of iron chelate to total chelate recovered is shown in Fig. 2. The values used for these diagrams are taken from Table I, except those

TABLE I

Analyses of soil extracts. Experiment 1. Mean values expressed as μg Fe. (Total chelate values in equivalents of iron)

...on chelate	Time of contact days	Treatment: 0.5 mg chelated iron (10 ppm Fe) — Soluble iron: Isotope method	Colorimetric method	Spectrophotometric	Total chelate	5.0 mg chelated iron (100 ppm Fe) — Soluble iron: Isotope method	Colorimetric method	Spectrophotometric	Total chelate
...e-EDTA	1	278	268	273	436	3220	3480	3520	4440
	2	245	242	246	410	2780	3000	2960	3830
	4	169	167	173	392	2170	2330	2320	3680
	8	81	89	108	406	1450	1570	1650	3590
	15*	79	100	113	403	1770	2020	2270	3590
...e-HEEDTA	1	158	158	182	323	2150	2170	2170	3030
	2	137	133	158	298	1850	1840	1920	2850
	4	89	95	125	313	1330	1390	1480	2530
	8	49	67	110	320	1120	1270	1420	2450+
	15*	26	54	96	398	900	1200	1410	2380

The treated soils for the 15-day interval were incubated in sealed containers.
+ Estimate of missing value.

TABLE II

Analyses of soil extracts. Experiment 2
Mean values, as μg Fe. (Total chelate values expressed in equivalents of iron)

Chelate treatment	Time of contact, days	Soluble iron (colorimetric method) Unsealed	Sealed	Soluble iron (spectrophotometric method) Unsealed	Sealed	Total chelate Unsealed	Sealed
Fe-EDTA	8	1450	2380	1540	2510	3840	4160
(5.0 mg Fe)	15	960	2120	1030	2300	3470	4110
Fe-HEEDTA	8	1260	1440	1450	1630	2970	2850
(5.0 mg Fe)	15	1080	1180	1300	1380	2720	2800

for the 15 day interval which come from the unsealed container results given in Table II. From Fig. 1 it will be seen that after an initial drop the total chelate recovery is almost constant at about 70% for Fe-EDTA and about 50% for Fe-HEEDTA. The soluble iron recovered as Fe-EDTA is high at first but falls continuously throughout the experiment: the initial amount of soluble Fe-HEEDTA is much lower but after 4 days remains almost constant.

The specific activity of the iron in the leachate compared with that of the chelate solution originally added to the soil, has been taken as a measure of the isotopic exchange that has taken place.

This exchange, calculated from the isotopically determined iron as a percentage of the iron determined colorimetrically (Table I), is shown in Fig. 3. It will be seen that, with both compounds, a higher

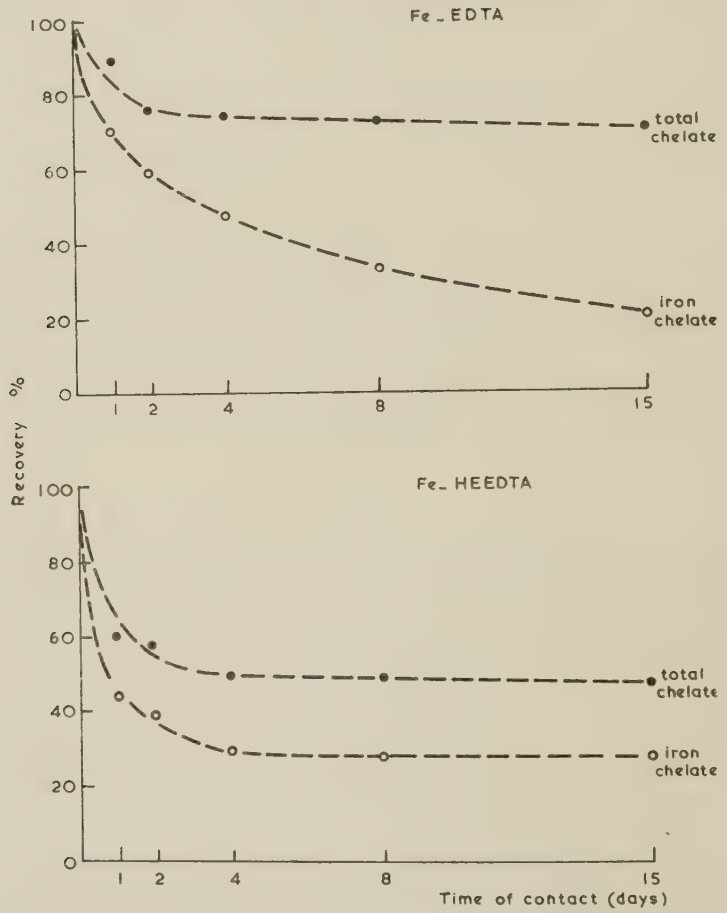

Fig. 1. Recovery of chelate from soil extracts. 100 ppm chelated iron incubated with 50 g calcareous soil under aerobic conditions.

proportion of the Fe^{59} has exchanged at the 10 ppm than at the 100 ppm treatment level. At corresponding levels of treatment Fe-HEEDTA has undergone isotopic exchange more rapidly than Fe-EDTA.

DISCUSSION

The general behaviour of both iron chelates can be attributed to two main causes. First there is a rapid sorption of chelate by the clay, the extent of which is not greatly affected by time, (Fig. 1).

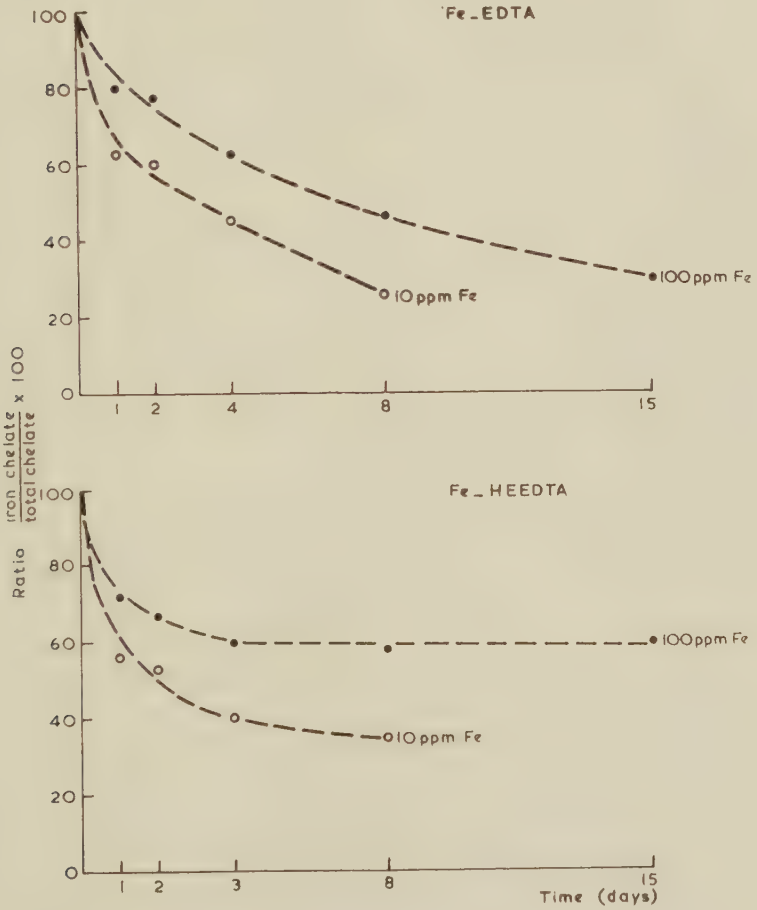

Fig. 2. Composition of total chelate present in soil extracts.

The second reaction, superimposed upon the first, is a loss of iron from the chelate, (Fig. 2), due to the substitution of calcium in the chelate molecule with subsequent precipitation of the iron. This

interpretation is in agreement with that of other workers (Stewart and Leonard [11], Wallace *et al.* [13]).

It is of interest to note in Table I that the percentage recovery of total chelate is of the same order from both the lower and the

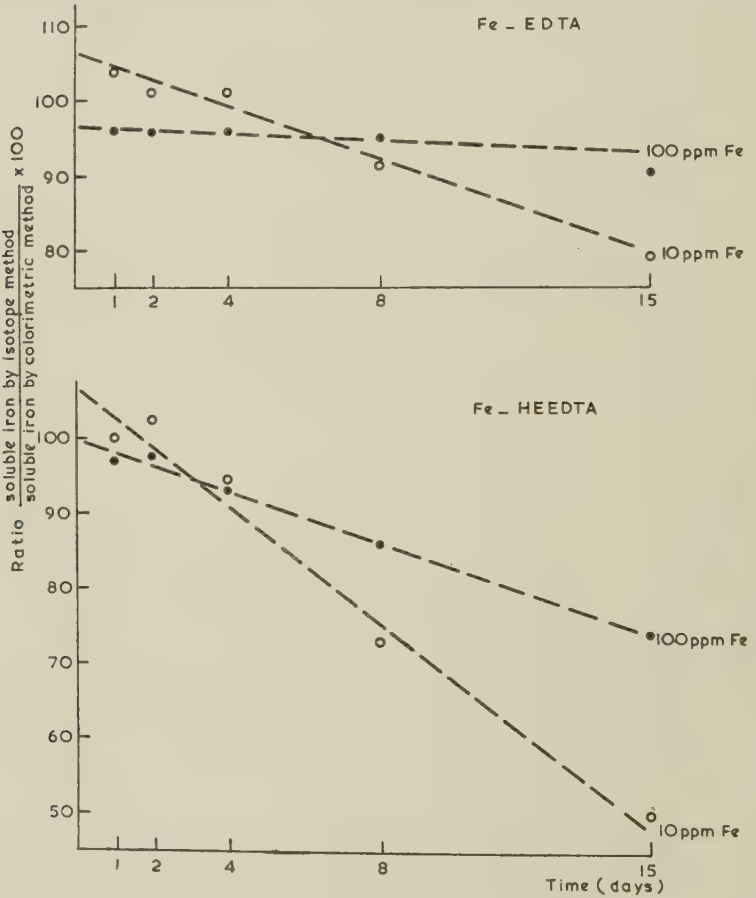

Fig. 3. Analyses of soil extracts, Experiment 1.

Ratio of $\frac{\text{soluble iron determined by isotope method}}{\text{soluble iron determined by colorimetric method}} \times 100.$

higher rates of application of EDTA and HEEDTA. It thus appears that the clay was not saturated with chelate anions at the lower treatment levels. Lunt and Wallace [8]) have also reported that

some clay minerals did not show changes in the percentage fixation with increasing application rates.

Our laboratory experiments show that initially more iron is kept in solution when it is applied as Fe-EDTA than as Fe-HEEDTA, there being a greater sorption of the latter by the clay. The rate of substitution of calcium for iron in the total chelate recovered is, however, much greater with EDTA than HEEDTA, so that after two weeks more soluble iron is present in the Fe-HEEDTA treatments. These results suggest that after the initial rapid reaction, the remaining Fe-HEEDTA is almost stable in calcareous soil. This long-term stability may be the reason for the better field results obtained with Fe-HEEDTA by Leonard and Stewart [7] and Wallace *et al.* [12].

The rapidity with which the amount of soluble iron decreases when these chelate solutions are applied to soil, is of great practical importance. Bould [1 2], and Wallace *et al.* [12], emphasize that on calcareous soils consistently favourable results can only be obtained by thoroughly watering the iron chelate into the soil or by sub-soil injection. Our results provide an explanation for this recommendation in that the most efficient method of using iron chelates in soil is to ensure that they reach the plant roots quickly, before the iron is precipitated, for it is as the chelate molecule itself that the iron is absorbed by the plant (Wallace *et al.* [14]). The inconsistent results reported by Leonard and Stewart [7] may well have been due to different methods of applying the treatment.

The restricted air supply to the soil during incubation with the chelate markedly increased the percentage of Fe-EDTA remaining in solution but had a much smaller effect on the total chelate recovery. No great difference was noted between the Fe-HEEDTA treatments, whether left with limited or with free access to the air. It is important, therefore, that in order to be comparable with field studies, laboratory experiments with Fe-EDTA in calcareous soil should be carried out under equivalent conditions of aeration. The reasons for this effect have not been investigated.

Table I shows that good agreement has been obtained between the analyses for iron in solution by the colorimetric and the ultra-violet spectrophotometric methods. The differences were greatest when the absorption due to the low concentration of iron chelate present was small in comparison with the "blank". After the longer

periods of contact with the soil the higher iron chelate figures may also be due to the presence of small amounts of chelated metals other than iron and calcium. It is recognised that errors due to the variation in the soil "blank" correction will also occur in the determination of the total chelate concentration by the method used in these experiments. But the reproducible results obtained and the ease and rapidity with which large numbers of these determinations can be made, were taken to justify its use here, in preference to the alternative colorimetric method (Darbey [3]).

In these experiments the same amount of radioactivity was used at each carrier level; this was considered permissible as isotopic exchange involves both radioactive and non-radioactive exchanges and will be independent of the concentration of tracer used (Norris [10]). Whilst the Fe^{59} has a slightly lower reactivity than the non-radioactive iron, largely Fe^{56}, this difference was considered to be negligible. McKay [9] and Norris [10] have pointed out that simple exchange reactions follow a first order rate law. When chelates are added to calcareous soil, however, sorption of the chelate by the clay occurs, and also replacement of iron by calcium, so for reasons of changing concentration and heterogeneity the first order rate law is unlikely to be followed. The experimental results given in Fig. 3 do not follow the first order rate law, but seem to be on a straight line when the specific activity of the extracts as percentages of the original specific activity are plotted against time. For both compounds isotopic exchange becomes more extensive when small amounts of labelled chelate are added to the soil. On this type of soil and at high levels of treatment, greater than 100 ppm Fe, isotopic exchange results in only a comparatively small loss of radioactivity from the Fe^{59}-labelled Fe-EDTA. It would appear therefore that provided the tracer experiment is not unduly prolonged, the exchange may be ignored, unless great accuracy is required of the results. However, the loss of Fe^{59} from Fe-HEEDTA is rapid even at the higher treatment level. When labelling any chelate with radioactive iron, the rate at which it will undergo isotopic exchange should be investigated first under the relevant experimental conditions, since failure to allow for exchange may lead to errors in the interpretation of experimental results.

Detailed laboratory studies, of the type reported here, on the behaviour of iron chelates in soil may well lead to more efficient use

of these compounds in their field application to the control lime-induced chlorosis.

SUMMARY

Fe-EDTA and Fe-HEEDTA, labelled with Fe^{59}, were applied at rates of 10 and 100 ppm iron to 50-g samples of dry soil, which were then stored for periods ranging from one to fifteen days before extracting with water.

The analyses of the extracts lead to the following general conclusions.

1. The decrease in soluble iron could be attributed to both sorption of chelate anions by the clay and to replacement of iron by calcium in the chelate molecule.

2. The amount of each chelate sorbed changed little with time and was proportional to the quantity applied. More HEEDTA was sorbed than EDTA.

3. The initial decrease in the concentration of soluble iron was rapid for both chelates, but was the greater for Fe-HEEDTA.

4. The precipitation of iron from the soluble Fe-HEEDTA was slower than from Fe-EDTA, so that fifteen days after treatment more soluble iron was obtained from the Fe-HEEDTA treatments.

5. Marked increases in the recoveries of Fe-EDTA were made when the treated soils were sealed within small containers, compared with those allowed free access to the air. Comparable treatments using Fe-HEEDTA had a much smaller effect.

6. Isotopic exchange of Fe^{59} with natural soil iron was greater in the treatments with 10 ppm chelated iron than the 100 ppm level. Fe-HEEDTA was subject to more exchange than Fe-EDTA at both levels.

These results are discussed in relation to the treatment of soils with iron chelates for the control of lime-induced chlorosis, and to the importance of isotopic exchange when using chelates labelled with radioactive iron in soil.

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PROBLEMS OF SOIL TESTING ON CALCAREOUS SOILS

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Chemically and mineralogically the soils of arid and semiarid regions are generally closely related to their native rocks. In many areas, because of the preponderance of limestone, most of the soils are calcareous. Extensive leaching may have removed the $CaCO_3$ from the soil, but often the amount of $CaCO_3$ in soils derived from calcareous parent material is considerable. Many loess soils or soils developed from soft limestone may contain 30 per cent or more of $CaCO_3$.

Such highly calcareous soils present special problems in soil analysis, partly due to the fact that most of the common soil testing methods have been developed in Europe, where highly calcareous soils are rare. The indiscriminate application of methods designed for acid soils to calcareous soils has often lead to a misunderstanding of the true properties of these soils.

Some of the problems associated with soil testing on calcareous soils have only recently been evaluated. Proper attention is now generally given to their particular properties in the course of the soil analysis. It is the object of this paper to discuss briefly some of the difficulties encountered and to indicate the means and ways used to overcome them.

SOIL REACTION

The determination of the pH of a soil is one of the simplest and most widely used soil tests. On non-calcareous soils many significant relationships have been obtained between the acidity of the soil and the degree of base saturation, nutrient release or fertility status. Small differences in pH on acid soils are usually of considerable

ecological significance, and it was possible to determine the optimal range of pH for most cultivated crops.

A similar significance is often erroneously ascribed to pH measurements on calcareous soils, without realising the implications associated with the presence of $CaCO_3$.

Pure calcium carbonate, when dissolved in carbon-dioxide-free water, hydrolizes to produce a solution with a pH of 9.95 (at 25°C). In the presence of carbon dioxide the alkalinity is reduced, and, depending on the partial pressure of CO_2 in the air, the pH of a saturated calcium carbonate solution may vary from 6.0 to 9.9.

From the sum of the three simultaneous equilibria of the system we obtain that

$$Ca^{++} + 2CO_2(l) + 2H_2O \leftrightharpoons CaCO_3 + 3H^+ + HCO_3^- \qquad (1)$$

The overall equilibrium constant of this reaction is the product of the equilibrium constants of the separate reactions:

$$\frac{[H^+]^3\,[HCO_3^-]}{[Ca^{++}]\,[CO_2]^2} = \frac{K^2_{H_2CO_3} \cdot K_{HCO_3^-}}{K_{CaCO_3}} \qquad (2)$$

If no extraneous ions are present the concentration of Ca^{++} and HCO_3^- is equivalent; then solving for pH we obtain:

$$pH = -\left[\tfrac{2}{3} \log P_{CO_2} + a\right] \qquad (3)$$

where P_{CO_2} is the partial pressure of carbon dioxide in atmospheres. The constant a is a function of all the participating simultaneous equilibria:

$$a = \tfrac{2}{3}\, pk_{CO_2} + \tfrac{2}{3}\, pK_{H_2CO_3} + \tfrac{1}{3}\, pK_{HCO_3^-} - \tfrac{1}{3}\, pK_{CaCO_3} \qquad (4)$$

and varies thus slightly with ionic strength and temperature. For 25°C and using the best available thermodynamic equilibrium constants the value of 5.90 is obtained *. The correction for ionic strength, for pressure up to one atmosphere carbon dioxide, when no other extraneous ions are present, is approximately 0.15 ± 0.02. The recalculation of available experimental data yielded a statistically higly significant constant equal to 6.04 (see Ref. [12] [13]).

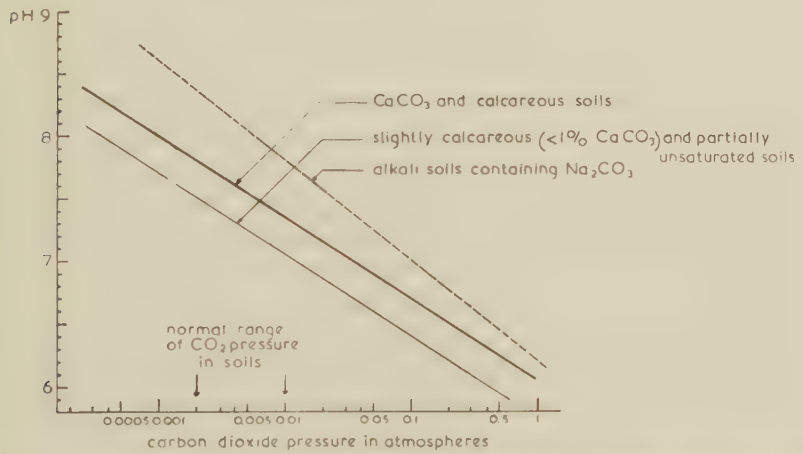
The effect of carbon dioxide on the pH of calcareous soils is

* The equilibrium constants (25°C) used in this and following calculations were: $pK_{H_2CO_3} = 6.37$, $pK_{HCO_3^-} = 10.33$, $pK_{CaCO_3} = 8.32$, $pk_{CO_2} = 1.47$, the latter being the solubility coefficient of carbon dioxide.

similar to that on the pH of pure carbonates. A calculation of the CO_2 function for the 18 calcareous soils studied by Whitney and Gardner [10] results in a regression coefficient and reaction constant practically identical with those obtained for pure $CaCO_3$:

$$pH = -0.65 \log P_{CO_2} + 6.00 \ (25°C). \qquad (5)$$

A more careful scrutiny of their data reveals three different levels of the function for three different types of soils (Fig. 1). Soils containing free alkalis have a slightly higher pH level (constant $a = 6.20$) and a somewhat steeper slope (regression coefficient $b = 0.8$), while partially unsaturated soils have a lower pH level ($a = 5.75$). The calcareous soils, low in sodium and magnesium, are intermediate and for all practical purposes identical with the pure salt.


pH 9
8
7
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$CaCO_3$ and calcareous soils
slightly calcareous (<1% $CaCO_3$) and partially unsaturated soils
alkali soils containing Na_2CO_3
normal range of CO_2 pressure in soils
0.0005 0.001 0.005 0.01 0.05 0.1 0.5 1
carbon dioxide pressure in atmospheres

Fig. 1. Effect of carbon dioxide pressure on the pH of pure $CaCO_3$ and of calcareous soils (25°C).

Atmospheric air has a fairly constant content of CO_2, about 0.03%, which imparts to a $CaCO_3$ solution a pH of 8.31. In the soil atmosphere the CO_2 pressure is somewhat higher, and generally very variable. It may reach values of up to 5% but varies mostly in the range of 0.2 — 1%. The pH of a calcareous soil will vary in a manner dependant upon by these limits of CO_2 pressure, and the regulating function of calcium ion concentration of the soil solution and its ionic strength.

Bjerrum and Gjaldbaek [3] were the first to show that the overall equilibrium condition of the $CaCO_3 - CO_2 - H_2O$ system is expressed by the equation:

$$pH - \tfrac{1}{2}\, pCa^{++} + \tfrac{1}{2} \log P_{CO_2} = \text{constant} \qquad (6)$$

The equilibrium constant is a function of the participating simultaneous equilibria:

$$\tfrac{1}{2}\,(pK_{H_2CO_3} + pK_{HCO_3^-} + pk_{CO_2} - pK_{CaCO_3}) \qquad (7)$$

and varies slightly with temperature and ionic strength. For 25°C the value 4.93 is obtained [12 13]. Equation 6 and 7 is obtained in a manner similar to equation 3 by the method of simultaneous equilibria, HCO_3^- having been eliminated from equation 1 and 2 by suitable substraction.

The correction for activities, when molar concentrations are used, can be calculated according to Debye-Hückel's theory. The theory leads us to expect that the ionic strength coefficient will lie close to 1.5, depending on the proportion of the dissociated mono- and divalent ions. By plotting the square root of ionic strength, $\sqrt{\mu}$, against the apparent equilibrium constant a straight line with a slope of 1.7 was obtained. In soils the contribution of exchangeable ions to the ionic strength is not easily evaluated. Yet the coefficient was found to give a satisfactory approximation for the conversion of $\log [Ca^{++}] - 1.7 \sqrt{\mu} = pCa^{++}$ in calcareous soils [13].

In Fig. 2 the pH of a saturated $CaCO_3$ solution is plotted as a function of the calcium ion concentration at the four levels of P_{CO_2} indicated. The calcium concentration corresponding to the solubility of gypsum is also indicated. The graphs demonstrate that pH of calcareous soils will generally vary in the range of pH 7.0—8.0, but may also reach pH 8.5. Measured values of pH within this range reflect therefore merely the CO_2 pressure and ionic strength at the time of measurement, and do not give any indication of the nature of the salts present in the soil solution. Only when the pH rises above 8.5 is there sufficient evidence that sodium carbonate or other strong alkali salts are present in the soil. If alkali salts are suspected in a soil with a pH lower than 8.5 other means for their characterization must be employed. If the pH falls below 7.0 it may indicate little or no $CaCO_3$ in the soil.

Slow equilibration and poor reproducibility of pH measurements

on calcareous soils is due to lack of control of CO_2 pressure. The CO_2 content of laboratory air is more variable than that of the outside atmosphere, and so is the CO_2 content of the distilled water used for suspending the soil, since this varies to some extent with temperature. Data obtained in the laboratory cannot therefore be directly related to field conditions, and difference between soils cannot be ecologically or physiologically interpreted. Values in the range of pH 7.0—8.5 provide no other information than that the soil may be calcareous.

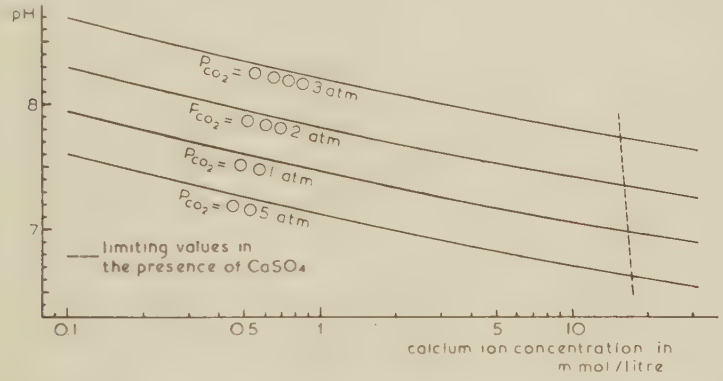

Fig. 2. pH of a saturated $CaCO_3$ suspension as a function of calcium ion concentration and carbon dioxide pressure (25°C).

Nevertheless, the measurements can be made significant if they are all carried out under standard conditions of both CO_2 pressure and calcium concentration. The soil minerals and the electrolytes present become then the only variables.

Exact control of CO_2 pressure is difficult to achieve and would not be practicable for routine measurements, but if the solution used for pH measurements is well aerated with atmospheric CO_2 and kept within a limited range of temperature, sufficiently constant conditions for practical purposes are obtained.

Workers at Rothamsted have recently suggested the use of 0.01 *M* $CaCl_2$ solution for pH measurements [9], and have shown, both on theoretical grounds and by experiments, that by controlling the electrolyte concentration in the suspension results may be obtained which are largely independent of dilution, and that steady and reproducible pH readings are recorded. In arid soils

it may be better to use a standard reagent of a somewhat higher electrolyte concentration. A saturated gypsum solution, being about 0.015 *M*, is admirably suited for this purpose in practical analysis. Due to the slightly higher calcium concentration the pH measurements in $CaSO_4$ show a somewhat lower pH value, wtih a maximal difference of 0.2 units between measurements in $CaCl_2$ and $CaSO_4$ solutions.

pH measurements on various types of calcareous soils from the subhumid to arid regions of Israel give values of 7.45—7.80 with $CaCl_2$ and 7.25—7.60 with $CaSO_4$ (Table I). A loess soil containing some soda has a pH of 8.0.

Routine analyses on a number of calcareous soils from England (containing more than 1% $CaCO_3$) show pH values between pH 7.2—7.8, narrowly grouped around the average of 7.5. Soils containing less than 1% $CaCO_3$ vary between 6.6—7.5, indicating that in such soils $CaCO_3$ is not evenly distributed, and that local unsaturation probably exists *.

From experience gained by pH measurements in $CaCl_2$ or $CaSO_4$ solutions it is evident that calcareous soils vary within narrow limits around pH 7.5, the value being somewhat higher in arid soils containing larger amounts of sodium salts.

In some experiments either sodium carbonate or sodium chloride was added to the soil suspension; with Na_2CO_3 the pH rose in all cases above 8.0, while the addition of neutral sodium salts produced only a small change in the pH. When saline conditions are suspected other methods, *e.g.* conductivity measurements, must thus be used for their identification and characterization.

At present very little is known about the optimal conditions of pH for plant growth on calcareous soils. It has been shown experimentally that the availability of several nutrients, *e.g.* iron and manganese, is greatly affected by the pH of the nutrient medium, but the interpretation under field conditions is greatly hampered by the lack of suitable data.

The very narrow range of pH obtained in calcareous soils seems to indicate that under field conditions they produce similar pH values under similar environmental conditions. Therefore, if for each soil type in question the variability of CO_2 under field conditions were

* Profile analyses by Soil Survey of England & Wales, presented at Field Meeting in Cambridge, 1955.

known, a fairly clear picture of the pH of the environment of the growing plant would emerge. The determination of the diurnal and seasonal variations of the CO_2 content in the soil atmosphere seems thus to be of considerable significance in the interpretation of the soil reaction on calcareous soils. As more data become available it may be possible to interpret differences between various calcareous soils also ecologically.

TABLE I

Some characteristic values of pH measurements on highly calcareous soils

Soil type *	Location	$CaCO_3$ %	pH by glass electrode		
			in aerated water	in 0.01 *M* $CaCl_2$	in saturated $CaSO_4$
Terra rossa	Jerusalem area	6.25	7.70	7.45	7.30
Rendzina	Bat Shlomo	7.10	7.60	7.45	7.25
Mountain marl	Safed	55.5	8.00	7.55	7.35
Lisan marl	Zemah	37.5	8.30	7.80	7.60
Black earth (Kabbara)	Ma'agan Michael	9.7	7.90	7.60	7.40
Loess soil **	Beersheba	23.8	8.80	8.00	8.00

* For description of these soils see Yaalon [11].

** Contains 6.5 me extractable Na per 100 gram soil.

MECHANICAL ANALYSIS

Another very common and widely used method for the characterization of soil properties is the mechanical analysis, which aims to give the relative proportions of the particle-size classes clay, silt and sand. The smaller particles are often cemented into variously sized aggregates, which are destroyed in the pretreatment of the soil. Calcium carbonate often acts as binding agent, and it has therefore been general practice to remove all $CaCO_3$ by HCl treatment as prescribed *e.g.* in the standard International Method of dispersing the soil. However, it is obvious that in calcareous soils where $CaCO_3$ may be as much as 30–50% of the total soil, not all of it acts as cementing agent. Most of it is actually present in the soil as distinct limestone, dolomite or calcite particles, possessing their own inherent grain size. A calcareous soil may therefore give an erroneous picture of its granulometric composition if the $CaCO_3$ is removed.

Various dispersing agents have been shown to enable a complete

dispersion of the soil even in the presence of considerable amounts of $CaCO_3$ and organic matter; *e.g.* sodium oxalate, sodium silicate, but especially the various polyphosphates, among which sodium polymethaphosphate, commercially known as Calgon, has found the widest application. When such dispersing agents are used, no other chemical pretreatment is necessary, except the removal of soluble salts if preliminary tests indicate that their concentration is high enough to flocculate the suspension.

The optimal concentration of the dispersing agent is best determined by preliminary tests; usually an amount equivalent to twice the cation exchange capacity is sufficient. No signs of coagulation should be observed after up to 24 hours standing. For serial analysis, using 10 g soil per liter, a 0.01 *N* solution with respect to sodium will be suitable in most cases. As the most stable suspensions are obtained at pH 8–10, some sodium carbonate which also serves to prevent the hydrolysis of the polyphosphate is added to the Calgon solution. When the size frequency is determined by the pipette method, which is still considered to be the most accurate method, a correction for the weight of the dispersion agent must be made.

A complete mechanical analysis is the basis for the textural classification of soils, and gives generally a very good indication of its physical properties. While the inclusion of $CaCO_3$ in the respective fractions is a considerable advance upon older methods, recent experience has shown that it is also desirable to know the particle size distribution of the calcareous material itself. In particular it appears to be important to know the amount of very fine calcium carbonate, which is believed to be responsible for the chlorosis of many crops. This latter information can be obtained either by granulometric analysis, *i.e.* by determining the amount of $CaCO_3$ in the separated fractions of clay and silt, or by adsorption methods, which will be discussed in the next chapter.

A complete particle size distribution of the calcareous material can be conveniently obtained by performing two mechanical analysis — one without and another with the removal of the lime by acid treatment. The higher percentage of a fraction when lime has not been removed during pretreatment is ascribed to the presence of $CaCO_3$. A direct determination of $CaCO_3$ in each fraction proved this assumption to be essentially correct, especially in highly calcareous soils. In some soils low in lime the acid treatment may

bring about an increase in the fraction consisting of particles less than 2μ equivalent spherical diameter. This increased amount of clay is likely due to the dispersion of aggregates cemented by lime and to the removal of calcareous coatings from silicate particles, but may also be due to a disintegration of some coarser particles by the action of the acid.

An example of the calculation of the size distribution of the calcareous material on two soils is given in Table II. From the analysis of the loess soil it can be seen that the granulometric distributions of the calcareous and siliceous materials are very similar, while in the Terra rossa soil there is practically no lime present in the fine fractions. Such complete analyses are therefore of significance in studies of soil formation and leaching rates on calcareous soils [11].

TABLE II

Calculation of the particle size distribution of the calcareous material from mechanical analysis. All values are expressed as percentages of oven-dry soil

Soil (Locality)	*	Size range of fraction				
		2000 200 μ %	200 20 μ %	20 2 μ %	< 2μ %	$CaCO_3$ %
Loess soil	A	1.9	68.0	13.8	17.1	
(Beersheba)	B	0.8	55.9	5.3	14.2	23.8
	A—B	0.3	12.1	8.5	2.9	
	C	1.3	50.8	35.7	12.2	
Terra rossa	A	1.7	21.4	17.7	59.2	
(Jerusalem)	B	0.3	17.7	17.2	59.2	6.2
	A—B	1.4	4.3	0.5	0.0	
	C	22.4	68.8	8.8	0.0	

* A = Particle size distribution of the untreated soil, including carbonates.
B = Acid-treated soil; particle size distribution of the non-carbonate minerals.
A–B = Distribution of the calcareous material.
C = Distribution of the calcareous material in % of total $CaCO_3$. C (A–B).100/percent $CaCO_3$.

SURFACE REACTIVITY OF LIME

Chlorosis of agricultural crops is often associated with highly calcareous soils. It is generally an iron deficiency symptom and is consequently cured or prevented by the application of soluble or chelated iron compounds, *e.g.* by leaf spraying.

The exact nature of the metabolic disturbances associated with

symptoms of iron deficiency on calcareous soils is still largely unknown. Toxic effects of locally high concentrations of bicarbonate ions have been suggested to be one of the main causes, but the reis also good evidence that the chlorotic symptoms are especially severe where the lime is present largely in the fine fraction of the soil [5 7].

A good measure of the potentiality of a calcareous soil to cause chlorosis is therefore obtained from a determination of the $CaCO_3$ in the clay and silt fraction, as described in the previous section. Based on the relation of particle size to surface area, which in turn governs the reactivity of solids and their rate of dissolution, the term "active lime" has been applied, to this fraction *. A convenient direct method for the determination of lime reactivity has been recently developed and its principles will be outlined in the following.

When a calcareous soil or a granular limestone is treated with a dilute oxalate solution the calcite surface becomes coated with precipitated calcium oxalate. If the rate of formation of calcium oxalate is taken to be a direct function of the reacting surface, a relative measure of lime reactivity is obtained by simply determining the amount of oxalate which reacted within a specific time. The method has been first used by Balks and Wehrmann [2] for agricultural lime, and adapted by Drouineau [5] for the diagnosis of chlorosis susceptibility on calcareous soils **.

The method as used by the author is a slight modification of the method used by French workers [4]. The details are as follows: 2 g of soil are treated for 2 hours with 200 ml 0.2 *N* NH_4-oxalate at pH 7.0, with occasional shaking. The soil is filtered and the amount of oxalate is determined in an aliquot of the filtrate by titration with 0.1 *N* $KMnO_4$. The amount of oxalate consumed is obtained as the difference between the original and final concentrations, and is expressed as a percentage or in me $CaCO_3$.

In general at reactivities higher than 10% the danger of chlorosis is considerable. Different crops, even different varieties of the same species, however, show variations in sensitivity; vines are generally

* The term "active" is somewhat misleading, as all of the lime in the soil is or may be active. "Reactivity" expresses more correctly the true conditions, and will be used henceforth. The relation between these two terms may be taken to be the same as in the analogous "soluble" and "solubility".

** Other reagents and reactions, *e.g.* rate of dissolution in carbonic acid or NH_4Cl, have also been used, but the oxalate reaction appears to be most convenient.

very sensitive while olives are highly resistant, As climatic and edaphic conditions influence the sensitivity of the crops, no generally applicable limits can be given, and these must therefore be determined for each region separately. The method as outlined must be considered as being semi-empirical, and as the following discussion will show, the results are greatly dependent on the experimental conditions. However, if standard conditions are maintained the reproducibility and sensitivity is good.

From considerations of the reactions taking place

$$CaCO_3\,(s) + C_2O_4^{--} + H_2O \leftrightharpoons CaC_2O_4\,(s) + HCO_3^- + OH^-$$

it can be concluded, from the relative solubilities of the two salts, that the reaction will proceed to the right until completion. The reaction ceases when all the oxalate is used up or when all available $CaCO_3$ has been converted to oxalate. From the equation it can be seen that as the reaction proceeds, the OH^- concentration of the solution will gradually rise. pH is therefore likely to affect the rate of the reaction.

Experimental evidence on the rate of reaction shows an initial very rapid rate followed by a much slower reaction, which continues for a long time but with a gradually diminishing rate. The initial rapid reaction can be attributed to the reactivity of the calcite surface, and proportionality to actual external surface has in fact been demonstrated by gas adsorption methods [8]. The slow secondary reaction may be ascribed to a less accessible inner surface.

The initial reaction is generally completed within a few minutes. A long time of contact is therefore of no particular advantage and in fact undesirable, except that the error of the time factor becomes less significant. If a time—reactivity curve is plotted, it is possible by extrapolation to separate the two reactions, which may be of interest in some special studies [6].

The rate of the reaction is greatly affected by the pH of the solution. If oxalic acid is used in the solution phase the reaction proceeds much more rapidly, mainly because the solubility of $CaCO_3$ is enhanced. With decreasing acidity it would be expected that the reactivity also decrease, still relatively high reactivity values may be obtained, apparently as a result of oxalate adsorption by the soil. But finally in the alkaline range the reactivity decreases due to the reduced $CaCO_3$ solubility. The relationship

between pH and reactivity tends therefore to be of a rather complex character, and it is also affected by dilution [1 4]. Some characteristic data obtained by the author are shown in Fig. 3, which shows clearly the effect of increased alkalinity on reactivity values.

The data demonstrate clearly the desirability of controlling the pH of the reacting solution; since the pH shifts during the reaction, only the final pH of the solution is of significance. In order to obtain comparable data it may be desirable to extrapolate all values to a common standard pH, in practice however, it is more convenient to buffer the solution by using a wide soil/solution ratio. But since the oxalate consumption is determined by difference, the accuracy of the determination is likely to suffer when wide soil/solution ratios are used. In general, therefore, best results are obtained in the range of pH 7—8 and with about 5—20% of the oxalate reacting. Thus if a 0.2 *N* oxalate solution is used, a ratio of 1 : 100 is suitable. The pH of the filtrate or the suspension is measured at the end of the reaction, and if it exceeds pH 8.0 the analysis is repeated with a higher soil/solution ratio.

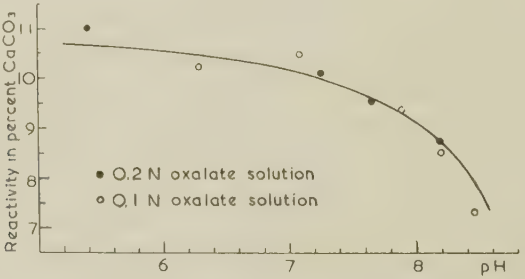

Fig. 3. Effect of pH on lime reactivity. (Loess soil, Beersheba, containing 23.8% $CaCO_3$. Soil/solution ratio 1 : 40. Time of reaction – overnight).

In soils, irrespective of the conditions of the reaction, the method is also subject to several systematic errors, which will be considered briefly.

Calcium from other sources than the surface of calcite, *e.g.* exchangeable Ca, will also become precipitated by the oxalate ions. Usually this error will be small; the maximum possible error can be easily calculated by assuming that all exchangeable calcium reacts. In some soils of a very high exchangeable-calcium content a suitable correction may be necessary. In gypsiferous soils the

error due to soluble Ca would be so large that the oxalate method is not applicable to them.

Another positive systematic error is caused by the adsorption of oxalate ions on clay mineral surfaces, mainly under alkaline conditions, while a negative error is caused by the dissolution of organic matter, which increases the permanganate consumption of the extract. Usually however both these errors are negligible.

The reactivity data are often expressed as weight or equivalents percent of $CaCO_3$. These may be convenient units in practice, but it should be always clearly understood that they essentially represent relative surface area. No causal relationship to total $CaCO_3$ can therefore be expected. So far no conversion factor has been derived which would transform the data into actual surface units. The thickness and uniformity of the oxalate layer appears to be too much dependent on the conditions of the reaction. Rigorous standardisation is necessary before a general correlation can be attempted. In spite of these shortcomings, the method is nevertheless extremely well suited for the estimation of the reactivity of $CaCO_3$ in soils and limestones, and provides us with an easily determinable relative measure of the reacting surface.

SUMMARY

The problems connected with soil testing on calcareous soils are discussed.

It is pointed out that in calcareous soils pH differences in the range of pH 7.0—8.5 are meaningless unless the measurements are made under standard conditions with respect to CO_2 and Ca-ion concentration. A knowledge of CO_2 variations under field conditions is considered essential for proper ecological interpretation of the reaction of calcareous soils.

It is stressed that the lime fraction in calcareous soils has its own inherent particle size distribution. Information regarding this fraction should therefore be included in the mechanical analysis of the soils. A method for the determination of the size frequency distribution of the $CaCO_3$ is outlined.

A simple method for the determination of the reactivity of the lime is described and the various conditions affecting the reaction are discussed. The method gives the relative surface area of the lime by oxalate adsorption and is used for the diagnosis of susceptibility to lime-induced chlorosis.

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